PN 4,868,112 Fee Code 111 Amount $1,120.00 INTERFERENCE

PATENT
Atty. Docket No.: 01142.0130

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 4,868,112)
)
Issued: September 19, 1989)
)
To: John J. Toole, Jr.)
)
Assignee: Genetics Institute, Inc.)
)
For: NOVEL PROCOAGULANT PROTEINS)

#16
5-24-00

BOX PATENT EXT.
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

APPLICATION FOR EXTENSION OF PATENT
TERM UNDER 35 U.S.C. § 156

Your Applicant, Genetics Institute, Inc., represents that it is the Assignee of the entire interest in and to Letters Patent of the United States No. 4,868,112 granted to John J. Toole, Jr. on the 19th day of September, 1989, for NOVEL PROCOAGULANT PROTEINS, by virtue of an assignment in favor of Genetics Institute, Inc. This assignment was recorded at the U.S. Patent and Trademark Office on Reel 4670, at frame 383, on December 9, 1986 (Attachment A).

By the Power of Attorney enclosed herein (Attachment B), Applicant appoints attorneys of Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., including Steven P. O'Connor, as attorney for Genetics Institute with regard to this application for

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extension of the term of U.S. Patent No. 4,868,112 and to transact all business in the U.S. Patent and Trademark Office in connection therewith.

Applicant hereby submits this application for extension of the patent term under 35 U.S.C. § 156 by providing the following information required by the rules promulgated by the U.S. Patent and Trademark Office (37 C.F.R. § 1.740). For the convenience of the Patent and Trademark Office, the information contained in this application is presented in a format that follows the requirements of Section 1.740 of Title 37 of the Code of Federal Regulations.

(1) The approved product, ReFacto®, is an antihemophilic factor for use in therapy for factor VIII deficiency comprising a purified protein produced by recombinant DNA technology. The formulation including ReFacto® is a sterile, nonpyrogenic, lyophilized preparation that contains a glycoprotein with an approximate molecular mass of 170 kDa consisting of 1438 amino acids comparable to the 90 + 80 kDa form of factor VIII.

(2) The approved product was subject to regulatory review under the Federal Food, Drug, and Cosmetic Act Section 505.

(3) The approved product ReFacto® received permission for commercial marketing or use under Section 505 of the Federal Food, Drug, and Cosmetic Act on March 6, 2000.

(4) The active ingredient in ReFacto® is a recombinant glycoprotein with an approximate molecular mass of 170 kDa consisting of 1438 amino acids comparable to the 90 + 80 kDa form of factor VIII, which, on information and belief, has not been

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approved for commercial marketing or use under Section 505 of the Federal Food, Drug, and Cosmetic Act before the approval of BLA 98-0137 for ReFacto® by the Food and Drug Administration on March 6, 2000. A copy of the insert describing the approved product is attached (Attachment C).

(5) This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the 60-day period pursuant to 37 C.F.R. § 1.720(f), said period will expire on May 4, 2000.

(6) The complete identification of the patent for which a term extension is being sought is as follows:

Inventor: John J. Toole, Jr.

Patent No.: 4,868,112

Issue Date: September 19, 1989

Expiration Date: September 19, 2006.

(7) A true copy of the patent is attached (Attachment D).

(8) No terminal disclaimer or reexamination certificate has been issued on this patent. A certificate of correction dated November 3, 1992, is attached (Attachment E). In addition, a copy of the maintenance fee statement indicating payment of maintenance fees on March 15, 1993, and March 19, 1997, is attached (Attachment F).

(9) U.S. Patent No. 4,868,112 claims a method of making the active ingredient in the approved product in claim 9, and the active ingredient in the approved product in claim 10. Claims 9 and 10 claim the active ingredient in ReFacto® as follows:

> 9. A method for producing a truncated Factor VIII:C protein which is an active procoagulant having the amino acid sequence of human Factor VIII:C but lacking at least 581 amino acids of the region between

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Arg-759 and Ser-1709 which comprises producing a genetically engineered mammalian host cell of claim 5 and culturing said host cell under condition permitting expression of the protein.

Claim 9 reads on the method of making the active ingredient in ReFacto® since it reads on a method of producing an active procoagulant having the amino acid sequence of human Factor VIII:C, but lacking amino acids 760-1667, by genetically engineering a Chinese hamster ovary (CHO) cell line and culturing that cell line such that it secretes B-domain deleted recombinant Factor VIII into the culture medium.

10. A truncated human Factor VIII:C protein which is an active procoagulant protein having a peptide sequence of human Factor VIII:C but lacking a peptide region selected from the group consisting of:

(a) the region between Pro-1000 and Asp-1582;
(b) the region between Thr-778 and Pro-1659; and
(c) the region between Thr-778 and Glu-1694.

Claim 10 reads on the active ingredient in ReFacto® since it reads on an active procoagulant having the amino acid sequence of human Factor VIII:C, but lacking amino acids 760-1667.

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(10) The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

Investigational New Drug Application (BB-IND 5348) for ReFacto® was filed November 30, 1993, and became effective on March 14, 1994, following removal of a clinical hold.

Biological License Application for ReFacto® (98-0137) was submitted on February 2, 1998.

Biological License Application for ReFacto® was approved on March 6, 2000.

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(11) A brief description of the significant activities undertaken by the marketing applicant and its collaborative partner during the applicable regulatory review period with respect to ReFacto® and the dates applicable to these significant activities are set forth in a chronology of events in Attachment G.

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(12)(I) Applicant is of the opinion that U.S. Patent 4,868,112 is eligible for extension of the patent term under 35 U.S.C. § 156 because it satisfies all requirements for such extension as follows:

(a) 35 U.S.C. § 156(a) - U.S. Patent 4,868,112 claims the product ReFacto®.

(b) 35 U.S.C. § 156(a)(1) - U.S. Patent 4,868,112 has not expired before submission of this application.

(c) 35 U.S.C. § 156(a)(2) - The term of U.S. Patent 4,868,112 has never been extended under 35 U.S.C. § 156(e)(1).

(d) 35 U.S.C. § 156(a)(3) - The application for extension is submitted by the owner of record of the patent in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d) and the rules of the Patent and Trademark Office.

(e) 35 U.S.C. § 156(a)(4) - The product ReFacto® has been subjected to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. § 156(a)(5)(A) - The commercial marketing or use of the product ReFacto® after the regulatory review period is the first permitted commercial marketing or use under the provision of the Federal Food, Drug and Cosmetic Act (that is, Section 505) under which such regulatory review period occurred.

(g) 35 U.S.C. § 156(c)(4) - No other patent has been extended for the same regulatory review period for the product ReFacto®.

(12)(ii) The length of the extension of patent term of U.S. Patent 4,868,112 claimed by Applicant is that period authorized by 35 U.S.C. § 156(c) which has been

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calculated to be 1475 days. The length of the extension was determined pursuant to 37 C.F.R. § 1.775 as follows:

(a) The regulatory review period under 35 U.S.C. § 156(g)(1)(B) began on March 14, 1994, and ended March 6, 2000, which is a total of 2186 days, which is the sum of (1) and (2) below:

(1) The period of review under 35 U.S.C. § 156(g)(1)(B)(I), the "Testing Period", began on March 14, 1994, and ended on February 2, 1998, which is 1422 days; and

(2) The period of review under 35 U.S.C. § 156(g)(1)(B)(ii), the "Approval Period", began on February 2, 1998, and ended on March 6, 2000, which is a total of 764 days.

(b) The regulatory review period upon which the period of extension is calculated is the entire regulatory review period as determined in subparagraph 12(ii)(a) above (2186 days) less:

(1) The number of days in the regulatory review period which were on or before the date on which the patent issued (September 19, 1989) which is zero (0) days; and

(2) The number of days during which applicant did not act with due diligence, which is zero (0) days; and

(3) One-half the number of days determined in sub-paragraph (12)(ii)(a)(1) above after the patent issued (one-half of 1422 days) which is 711 days;

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(c) The number of days as determined in sub-paragraph (12)(ii)(b) (1475 days) when added to the expiration date of the original term of the patent (September 19, 2006) would result in the date of October 3, 2010.

(d) Fourteen (14) years when added to the date of the BLA approval (March 6, 2000) would result in the date of March 6, 2014;

(e) The earlier date as determined in sub-paragraphs (12)(ii)(c) and (12)(ii)(d) is October 3, 2010;

(f) Since U.S. Patent 4,868,112 issued after September 24, 1984, the period of extension may not exceed five years from the original expiration date of September 19, 2006. Five years when added to the original expiration date of the patent would result in the date of September 19, 2011.

(g) The earlier date as determined by sub-paragraphs (12)(ii)(e) and (12)(ii)(f) is October, 3, 2010.

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

While Applicant does not consider the information to be material to the determination of entitlement to the extension sought, it points out that U.S. Patent No. 4,868,112 is currently the subject of interference no. 103,215. Claim 9 is designated as corresponding to count 1 of this interference, while claim 10 is designated as corresponding to count 2.

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(14) The prescribed fee for receiving and acting upon this application is attached as a check in the amount of $1,120.00. The Commissioner is authorized to charge any additional fees required by this application to Deposit Account No. 06-0916.

(15) All correspondence and inquiries may be direct to the undersigned, whose address, telephone number, and fax number are as follows:

Steven P. O'Connor
Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.
1300 I Street, N.W.
Washington, D.C. 20005-3315

Phone: 202-408-4079
Fax: 202-408-4400

(16) Enclosed is a certification that the application for extension of patent term under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and four (4) copies thereof (Attachment H).

(17) The requisite declaration pursuant to 37 C.F.R. § 1.740(b) is attached (Attachment I).

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: Steven P. O'Connor
Steven P. O'Connor
Reg. No. 41,225

Date: May 4, 2000

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Attachments:
Assignment (Attachment A)
Power of Attorney (Attachment B)
Package Insert for ReFacto® (Attachment C)
U.S. Patent No. 4,868,112 (Attachment D)
Copy of Certificate of Correction (Attachment E)
Copy of Maintenance Fee Statement (Attachment F)
Chronology of Regulatory Review Period (Attachment G)
Certification of Copies of Application Papers (Attachment H)
Declaration Pursuant to 37 C.F.R. § 1.740(b) (Attachment I)

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PATENT NUMBER: 4868112 ISSUE DATE: 09/19/89
SERIAL NUMBER: 07/010085 FILING DATE: 04/11/86
PCT NUMBER: PCT/US86/00774 PCT DATE: 04/11/86
TITLE: NOVEL PROCOAGULANT PROTEINS
APPLICANT: TOOLE, JOHN J.JR.

REEL: 004670 FRAME: 0383 DATE RECORDED: 12/09/86 NUMBER OF PAGES: 002
ASSIGNOR: TOOLE, JOHN J. JR.
EXC DATE: 04/10/86
ASSIGNEE: GENETICS INSTITUTE, INC., 87 CAMBRIDGE PARK DRIVE, MASSACHUS
ETTS A CORP. OF DE.
BRIEF: ASSIGNMENT OF ASSIGNORS INTEREST.
RETURN ADDRESS: DAVID L. BERSTEIN
C/O GENETICS INSTITUTE, INC.
87 CAMBRIDGE PARK DRIVE
CAMBRIDGE, MA 02140-2387

NO MORE INFORMATION FOR THIS PATENT NUMBER 04/27/00 15:32

Assignment

In consideration of good and valuable considerations, the receipt of which is hereby acknowledged, I , the undersigned,

John J. Toole, Jr., residing at
27 Lakeville Road, Jamaica Plain, Massachusetts 02140

Hereby sell, assign and transfer to Genetics Institute, Inc.
a corporation of the State of Delaware having a place of business at 87 CambridgePark Drive, Cambridge in the County of Middlesex and State of Massachusetts its successors, assigns and legal representatives, the entire right, title and interest for all countries, in and to any and all inventions which are disclosed and claimed, and any and all inventions which are disclosed but not claimed, in the application for United States Patent, which has been executed by the undersigned on 10 April 1986 and is entitled

REEL 4670 FRAME 383

NOVEL PROCOAGULANT PROTEINS
(a continuation-in-part of U.S. Serial No. 725,350 filed April 12, 1985)

and in and to said application and all divisional, continuing, substitute, renewal, reissue, and all other applications for U.S. Letters Patent or other related property rights in any and all foreign countries which have been or shall be filed on any of said inventions disclosed in said application; and in and to all original and reissued patents or related foreign documents which have been or shall be issued on said inventions;

Authorize and request the Commissioner of Patents of the United States to issue to said Assignee, the corporation above named, its successors, assigns and legal representatives, in accordance with this assignment, any and all United States Letters Patent on said inventions or any of them disclosed in said application;

Agree that said Assignee may apply for and receive foreign Letters Patent or rights of any other kind for said inventions, or any of them; and may claim, in applications for said foreign Letters Patent or other rights, the priority of the aforesaid United States patent application under the provisions of the International Convention of 1883 and later modifications thereof, under the Patent Cooperation Treaty, under the European Patent Convention or under any other available international agreement; and that, when requested, without charge to, but at the expense of, said Assignee, its successors, assigns and legal representatives, to carry out in good faith the intent and purpose of this assignment, the undersigned or the undersigned's executors or administrators will, for the United States and all foreign countries, execute all divisional, continuing, substitute, renewal, reissue, and all other patent applications or other documents on any and all said inventions; execute all rightful oaths, assignments, powers of attorney and other papers; communicate to said Assignee, its successors, assigns and representatives, all facts known and documents available to the undersigned relating to said inventions and the history thereof; testify in all legal proceedings; and generally do everything possible which said Assignee, its successors, assigns or representatives shall consider desirable for aiding in securing, maintaining and enforcing proper patent protection for said inventions and for vesting title to said inventions and all applications for patents or related foreign rights and all patents on said inventions, in said Assignee, its successors, assigns and legal representatives; and

Covenant with said Assignee, its successors, assigns and legal representatives that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by the undersigned, and that full right to convey the same as herein expressed is possessed by the undersigned.

John J. Toole Jr. [L.S.]
John J. Toole, Jr.

Before me this 10th day of April, 1986 personally appeared John J. Toole, Jr. who is known to me personally, and asknowledged the foregoing instrument of assignment to be his free act and deed.

Teresa Weil

TERESA WEIL
NOTARY PUBLIC
MY COMMISSION EXPIRES
NOV. 14 1991

[L.S.]

RECORDED
PATENT & TRADEMARK OFFICE
DEC -9 1986

REEL 4670 FRAME 384

PATENT
Atty. Docket No.: 01142.0130

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 4,868,112)
)
Issued: September 19, 1989)
)
To: John J. Toole, Jr.)
)
Assignee: Genetics Institute, Inc.)
)
For: NOVEL PROCOAGULANT PROTEINS)

BOX PATENT EXT.
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

POWER OF ATTORNEY

Genetics Institute, Inc., is the Assignee of the entire right, title, and interest in the patent identified above by virtue of an assignment recorded in the Patent and Trademark Office at Reel 4670, at frame 383, on December 9, 1986

Assignee, Genetics Institute, Inc., being the owner of the above-identified U.S. Letters Patent, hereby grants power of attorney to **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.,** Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C.

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Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387; Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Barry W. Graham, Reg. No. 29,924; Susan Haberman Griffen, Reg. No. 30,907; Richard B. Racine, Reg. No. 30,415; Thomas H. Jenkins, Reg. No. 30,857; Robert E. Converse, Jr., Reg. No. 27,432; Clair X. Mullen, Jr., Reg. No. 20,348; Christopher P. Foley, Reg. No. 31,354; John C. Paul, Reg. No. 30,413; Roger D. Taylor, Reg. No. 28,992; David M. Kelly, Reg. No. 30,953; Kenneth J. Meyers, Reg. No. 25,146; Carol P. Einaudi, Reg. No. 32,220; Walter Y. Boyd, Jr., Reg. No. 31,738; Steven M. Anzalone, Reg. No. 32,095; Jean B. Fordis, Reg. No. 32,984; Barbara C. McCurdy, Reg. No. 32,120; James K. Hammond, Reg. No. 31,964; Richard V. Burgujian, Reg. No. 31,744; J. Michael Jakes, Reg. No. 32,824; Thomas W. Banks, Reg. No. 32,719; Christopher P. Isaac, Reg. No. 32,616; Bryan C. Diner, Reg. No. 32,409; M. Paul Barker, Reg. No. 32,013; Andrew Chanho Sonu, Reg. No. 33,457; David S. Forman, Reg. No. 33,694; Vincent P. Kovalick, Reg. No. 32,867; James W. Edmondson, Reg. No. 33,871; Michael R. McGurk, Reg. No. 32,045; Joann M. Neth, Reg. No. 36,363; Gerson S. Panitch, Reg. No. 33,751; Cheri M. Taylor, Reg. No. 33,216; Charles E. Van Horn, Reg. No. 40,266; Linda A. Wadler, Reg.

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No. 33,218; Jeffrey A. Berkowitz, Reg. No. 36,743; Michael R. Kelly, Reg. No. 33,921; and James B. Monroe, Reg. No. 33,971; and Steven P. O'Connor, Reg. No. 41,225, both jointly and separately to be attorneys for Genetics Institute with regard to an application for extension of the term of U.S. Patent No. 4,868,112 and to transact all business in the Patent and Trademark Office connected therewith.

The undersigned is empowered to act on behalf of the Assignee.

Please send all future correspondence concerning the above matter to Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., at the following address:

Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.
1300 I Street, N.W.
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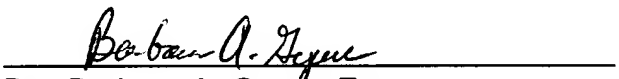
GENETICS INSTITUTE, INC.



By: Barbara A. Gyure, Esq.
Assistant Secretary
Reg. No. 34,614

Date: May 2, 2000

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ReFacto®

Antihemophilic Factor, Recombinant

DESCRIPTION

ReFacto® Antihemophilic Factor (Recombinant) is a purified protein produced by recombinant DNA technology for use in therapy of factor VIII deficiency. ReFacto is a glycoprotein with an approximate molecular mass of 170 kDa consisting of 1438 amino acids. It has an amino acid sequence that is comparable to the 90 + 80 kDa form of factor VIII, and post-translational modifications that are similar to those of the plasma-derived molecule. ReFacto has *in vitro* functional characteristics comparable to those of endogenous factor VIII.

ReFacto is produced by a genetically engineered Chinese hamster ovary (CHO) cell line. The CHO cell line secretes B-domain deleted recombinant factor VIII into a defined cell culture medium that contains human serum albumin and recombinant insulin, but does not contain any proteins derived from animal sources. The protein is purified by a chromatography purification process that yields a high-purity, active product. The potency expressed in International units (IU) is determined using the European Pharmacopoeial chromogenic assay against the WHO standard. The specific activity of ReFacto is 11,200-15,500 IU per milligram of protein. ReFacto is not purified from human blood and contains no preservatives or added human components in the final formulation.

ReFacto is formulated as a sterile, nonpyrogenic, lyophilized powder preparation for intravenous (IV) injection. It is available in single-use vials containing the labeled amount of factor VIII activity (IU). Each vial contains nominally 250, 500, or 1000 IU of ReFacto per vial. The formulated product is a clear colorless solution upon reconstitution and contains sodium chloride, sucrose, L-histidine, calcium chloride, and polysorbate 80.

CLINICAL PHARMACOLOGY

Factor VIII is the specific clotting factor deficient in patients with hemophilia A (classical hemophilia). The administration of ReFacto® Antihemophilic Factor (Recombinant) increases plasma levels of factor VIII activity and can temporarily correct the *in vitro* coagulation defect in these patients.

Activated factor VIII acts as a cofactor for activated factor IX accelerating the conversion of factor X to activated factor X. Activated factor X converts prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin and a clot is formed. Factor VIII activity is greatly reduced in patients with hemophilia A and therefore replacement therapy is necessary.

In a crossover pharmacokinetic study of eighteen (18) previously treated patients using the chromogenic assay, the circulating mean half-life for ReFacto was 14.5 ± 5.3 hours (ranged from 7.6-27.7 hours), which was not statistically significantly different from plasma-derived Antihemophilic Factor (Human) (pdAHF), which had a mean half-life of 13.7 ± 3.4 hours (ranged from 8.8-23.7 hours). Mean incremental recovery (K-value) of ReFacto in plasma was 2.4 ± 0.4 IU/dL per IU/kg (ranged from 1.9 - 3.3 IU/dL per IU/kg). This was comparable to the mean incremental recovery observed in plasma for pdAHF which was 2.3 ± 0.3 IU/dL per IU/kg (ranged from 1.7 - 2.9 IU/dL per IU/kg). Results obtained from this controlled pharmacokinetic study, which used a central laboratory for the analysis of all plasma samples, showed that the one-stage factor VIII clotting assay gave results which were approximately 50% of the values obtained with the chromogenic assay.

In two additional clinical studies, pharmacokinetic parameters were evaluated for previously treated patients (PTPs) and previously untreated patients (PUPs). In PTPs (n=87) ReFacto had a mean incremental recovery of 2.4 ± 0.4 IU/dL per IU/kg (ranged from 1.1-3.8 IU/dL per IU/kg) and an elimination half-life (n=67) of 10.7 ± 2.8 hours. In PUPs (n=45) ReFacto had a lower mean incremental recovery of 1.7 ± 0.4 IU/dL per IU/kg (ranged from 0.2-2.8 IU/dL per IU/kg) as compared to PTPs. Population pharmacokinetic modeling using data from 44 PUPs led to a mean estimated half-life of ReFacto in PUPs of 8.0 ± 2.2 hours. These parameters did not change over time (12 months) for PTPs or PUPs.

In clinical studies of ReFacto involving a total of 218 patients (117 PTPs including 4 who participated in the surgery study only, and 101 PUPs), more than 84 million IU were administered over a period of up to 54 months. The 117 PTPs were given a median of 230 injections (range of 4-1530 injections) over a median of 1200 days (range of 31-1640 days). The 101 PUPs were given a median of 26 injections (range of 1-490 injections) over a median of 830 days (range of 1-1298 days). One hundred thirteen PTPs and 99 PUPs were evaluated for efficacy in bleeding episodes. The 113 PTPs experienced a median of 54 bleeding episodes and the 99 PUPs experienced a median of 12 bleeding episodes. All were treated successfully on an on-demand basis or for the reduction of bleeding episodes except for one PTP and two PUPs who discontinued ReFacto treatment and switched to another product after the development of inhibitors. Bleeding episodes included hemarthroses, and bleeding in soft tissue, muscle, and other anatomical sites.

ReFacto has been studied in short-term routine prophylaxis. In uncontrolled clinical trials, an average dose of 27±10 IU/kg in PTPs (n=77) and an average dose of 57±20 IU/kg in PUPs (n=17) was given repeatedly at variable intervals longer than 2 weeks. In 64 patients who had both on-demand and prophylactic periods during their time on study, the mean rate of spontaneous musculoskeletal bleeding episodes was less during periods of routine prophylaxis. There were an average of 10 bleeding episodes per year during the prophylactic periods compared to an average of 37 bleeding episodes per year during the on-demand periods. The clinical trial experience with routine prophylaxis in PUPs is limited (n=17). These non-randomized trial results should be interpreted with caution, as the investigators exercised their own discretion in deciding when and in whom prophylaxis was to be initiated and terminated.

Management of hemostasis was evaluated in the surgical setting where 28 surgical procedures have been performed in 25 patients. The average preoperative dose in PTPs was 59 IU/kg. Procedures included orthopedic procedures, inguinal hernia repair, epidural hematoma evacuation, transposition ulnar nerve, and other minor procedures (e.g., venous access catheter placement and explantation, toenail removal). Circulatory factor VIII levels targeted to restore and maintain hemostasis were achieved. While the one-stage clotting assay was used most frequently in the surgical setting (24 versus 4 surgeries), hemostasis was maintained throughout the surgical period regardless of which assay was used. Hemostatic efficacy was rated as excellent or good in all procedures.

The occurrence of neutralizing antibody (inhibitors) is well known in the treatment of patients with hemophilia A[1,2]. Thirty out of 101 PUPs (30%) developed an inhibitor: 16 out of 101 (16%) with a high titer (≥ 5 BU) (11 of the 16 patients had peak values ≥10 BU/mL) and 14 out of 101(14%) with a low titer (< 5 BU). In this study the incidence of inhibitor development to factor VIII using ReFacto is similar to that reported for other factor VIII products[1,2].

One of 113 PTPs (0.9%) developed a low titer inhibitor after 107 exposure days with ReFacto. In this study the incidence of inhibitor development to factor VIII using ReFacto is similar to that reported for other factor VIII products[4].

INDICATIONS AND USAGE

ReFacto® Antihemophilic Factor (Recombinant) is indicated for the control and prevention of hemorrhagic episodes and for surgical prophylaxis in patients with hemophilia A (congenital factor VIII deficiency or classic hemophilia).

ReFacto is indicated for short-term routine prophylaxis to reduce the frequency of spontaneous bleeding episodes. The effect of regular routine prophylaxis on long-term morbidity and mortality is unknown.

ReFacto can be of a significant therapeutic value for treatment of hemophilia A in certain patients with inhibitors to factor VIII[5]. In clinical studies of ReFacto, patients who developed inhibitors on study continued to manifest a clinical response when inhibitor titers were < 10 BU/mL. When an inhibitor is present, the dosage requirement of factor VIII is variable. The dosage can be determined only by a clinical response and by monitoring of circulating factor VIII levels after treatment (see **DOSAGE AND ADMINISTRATION**).

ReFacto does not contain von Willebrand factor and therefore is not indicated in von Willebrand's disease.

CONTRAINDICATIONS

Known hypersensitivity to mouse, hamster, or bovine proteins may be a contraindication to the use of ReFacto® Antihemophilic Factor (Recombinant).

WARNINGS

As with any intravenous protein product, allergic type hypersensitivity reactions are possible. Patients should be informed of the early signs of hypersensitivity reactions including hives, generalized urticaria, tightness of the chest, wheezing, hypotension, and anaphylaxis. Patients should be advised to discontinue use of the product and contact their physicians if these symptoms occur.

PRECAUTIONS

General

Activity-neutralizing antibodies (inhibitors) have been detected in patients receiving factor VIII-containing products. There is no evidence that ReFacto® Antihemophilic Factor (Recombinant) is associated with a higher-than-historical incidence of inhibitors. As with all coagulation factor VIII products, patients should be monitored for the development of inhibitors that should be titrated in Bethesda Units using appropriate biological testing.

Formation of Antibodies to Mouse and Hamster Protein

As Antihemophilic Factor (Recombinant), ReFacto contains trace amounts of mouse protein (maximum of 5 ng/1000 IU) and hamster protein (maximum of 30 ng/1000 IU), the remote possibility exists that patients treated with this product may develop hypersensitivity to these non-human mammalian proteins.

Carcinogenicity, Mutagenicity, Impairment of Fertility

ReFacto® Antihemophilic Factor (Recombinant) has been shown to be nonmutagenic in the mouse micronucleus assay. No other mutagenicity studies and no investigations on carcinogenesis or impairment of fertility have been conducted.

Pregnancy Category C

Animal reproduction and lactation studies have not been conducted with ReFacto® Antihemophilic Factor (Recombinant). It is not known whether ReFacto can affect reproductive capacity or cause fetal harm when given to pregnant women. ReFacto should be administered to pregnant and lactating women only if clearly indicated.

Pediatric Use

ReFacto® Antihemophilic Factor (Recombinant) is appropriate for use in children of all ages, including newborns. Safety and efficacy studies have been performed both in previously treated children and adolescents (N=22, ages 8-15 years) and in previously untreated neonates, infants, and children (N=101, ages 0-52 months) (see **CLINICAL PHARMACOLOGY** and **PRECAUTIONS**).

Geriatric Use

Clinical studies of ReFacto did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. As with any patient receiving ReFacto, dose selection for an elderly patient should be individualized.

ADVERSE REACTIONS

As with the intravenous administration of any protein product, the following reactions may be observed after administration: headache, fever, chills, flushing, nausea, vomiting, lethargy, or manifestations of allergic reactions. During clinical studies with ReFacto® Antihemophilic Factor (Recombinant), 77 adverse reactions in 43 of 218 patients (20%) probably or possibly related to therapy were reported for 64,363 infusions (0.12%). These were anaphylaxis (1), dyspnea (6), urticaria (1), nausea (11), headache (4), vasodilation (5), dizziness (4), permanent venous access catheter complications (3), asthenia (3), fever (3), taste perversion (altered taste) (3), bleeding/hematoma (3), infected hematoma (1), anorexia (2), diarrhea (2), injection site reaction (2), somnolence (2), rash (2), pruritus (2), angina pectoris (1), tachycardia (1), perspiration increased (1), chills (1), increased amino transferase (1), increased bilirubin (1), pain in finger (1), muscle weakness (1), CPK increase (1), cold sensation (1), eye disorder-vision abnormal (1), coughing (1), myalgia (1), gastroenteritis (1), abdominal pain (1), acne (1), and forehead bruises (1). If any adverse reaction takes place that is thought to be related to administration of ReFacto, the rate of infusion should be decreased or stopped.

In addition, inhibitor development is a known adverse event associated with the treatment of patients with hemophilia A (see **CLINICAL PHARMACOLOGY**).

A total of 182 adverse reactions in 54 of 218 patients (25%) who received 32,013 infusions (0.6%) were reported by the investigator to have an "unlikely" or "not assessable" relationship to ReFacto administration. The study sponsor considered that the events may be of possible or of unknown relationship to therapy because of the temporal relationship to the infusion and/or the frequency of the event for a given patient and/or because insufficient information was available to assign another causality. In this category, 25 patients experienced the following 38 events which are different from the events described above: pain (10), rhinitis (10), vomiting (4), insomnia (3), constipation (2), pharyngitis (2), flushing (1), palpitation (1), sinusitis (1), gastritis (1), dyspepsia (1), hypotension (1), and URI (1).

Other adverse experiences that were reported during the clinical trials, but which were assessed by both the investigator and the sponsor as "unlikely" to be related to ReFacto administration included: dyspnea (3), rash (2), pruritus (1), neuropathy (1), arm weakness (1), and thrombophlebitis of upper arm (1).

DOSAGE AND ADMINISTRATION

Treatment with ReFacto® Antihemophilic Factor (Recombinant) should be initiated under the supervision of a physician experienced in the treatment of hemophilia A.

Dosage and duration of treatment depend on the severity of the factor VIII deficiency, the location and extent of bleeding, and the patient's clinical condition. Doses administered should be titrated to the patient's clinical response. In the presence of an inhibitor, higher doses may be required.

One international unit (IU) of factor VIII activity corresponds approximately to the quantity of factor VIII in one mL of normal human plasma. The calculation of the required dosage of factor VIII is based upon the empirical finding that, on average, 1 IU of factor VIII per kg body weight raises the plasma factor VIII activity by approximately 2 IU/dL per IU/kg administered. The required dosage is determined using the following formula:

Required units = body weight (kg)
× desired factor VIII rise (IU/dL or % of normal)
× 0.5 (IU/kg per IU/dL)

The following chart can be used to guide dosing in bleeding episodes and surgery:

Type of Hemorrhage	Factor VIII Level Required (IU/dL or % of normal)	Frequency of Doses (h)/ Duration of Therapy (d)
Minor		
Early hemarthrosis, minor muscle or oral bleeds.	20–40	Repeat every 12 to 24 hours as necessary until resolved. At least 1 day, depending upon the severity of the hemorrhage.
Moderate		
Hemorrhages into muscles. Mild trauma capitis. Minor operations including tooth extraction. Hemorrhages into the oral cavity.	30–60	Repeat infusion every 12–24 hours for 3–4 days or until adequate local hemostasis is achieved. For tooth extraction a single infusion plus oral antifibrinolytic therapy within 1 hour may be sufficient.
Major		
Gastrointestinal bleeding. Intracranial, intra-abdominal or intrathoracic hemorrhages. Fractures. Major operations.	60–100	Repeat infusion every 8–24 hours until the bleed is resolved or in the case of surgery, until adequate local hemostasis is achieved.

Precise monitoring of the replacement therapy by means of coagulation analysis (plasma factor VIII activity) is recommended, particularly for surgical intervention.

Product is labeled on the basis of the chromogenic assay. The available clinical trial data suggest either the one-stage clotting assay or the chromogenic assay may be used to help follow patients clinically. Most clinical trial subjects were monitored with the one-stage clotting assay. It must be noted that the one-stage clotting assay yields results which are lower than the values obtained with the chromogenic assay (see **CLINICAL PHARMACOLOGY**).

For short-term routine prophylaxis to prevent or reduce the frequency of spontaneous musculoskeletal hemorrhage in patients with hemophilia A, ReFacto should be given at least twice a week. In some cases, especially pediatric patients, shorter dosage intervals or higher doses may be necessary. Pharmacokinetic/pharmacodynamic modeling, based on pharmacokinetic data from 185 infusions in 102 PTPs, predicts that routine prophylactic dosing 3 times per week may be associated with a lower bleeding risk than with dosing twice weekly. No randomized comparison of different doses or frequency regimens of ReFacto for routine prophylaxis has been performed. In clinical studies in PTPs (ages 8-73 years) and PUPs (ages 9-52 months), the mean dose used for routine prophylaxis was 27 ± 10 IU/kg and 57 ± 20 IU/kg, respectively.

Patients using ReFacto should be monitored for the development of factor VIII inhibitors. If expected factor VIII activity plasma levels are not attained, or if bleeding is not controlled with an appropriate dose, an assay should be performed to determine if a

United States Patent [19]

Toole, Jr.

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[54] **NOVEL PROCOAGULANT PROTEINS**

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[63] Continuation-in-part of Ser. No. 725,350, Apr. 12, 1985, abandoned.

[51] **Int. Cl.**[4] **C12P 21/00**; C12P 21/02; C12N 15/00; C07H 15/12

[52] **U.S. Cl.** **435/68**; 435/70; 435/172.3; 435/240.1; 435/240.2; 435/320; 435/948; 435/252.33; 530/383; 536/27; 514/2; 514/8

[58] **Field of Search** 435/68, 70, 172.3, 253, 435/255, 256, 240.1, 240.2, 320; 530/383; 534/27; 935/11, 32, 34, 56, 57, 60, 70; 514/2, 8

[56] **References Cited**

PUBLICATIONS

Wood et al, *Nature*, vol. 312, 22 Nov. 1984, pp. 330–336, "Expression of Active Human Factor VIII from Recombinant DNA Clones".

Toole et al, *Nature*, vol. 312, 22 Nov. 1984, pp. 342–347, "Molecular Cloning of a cDNA Encoding Human Antihaemophilic Factor".

Vehar et al, *Nature*, vol. 312, 22 Nov. 1984, pp. 337–342, "Structure of Human Factor VIII".

Orr et al, *Thrombos Haemostasis*, vol. 57(1), p. 57, 1985, "Spacer Function Imp for the Heavily Glycosylated Region of Factor VIII".

Toole et al, *Proc. Natl. Acad. Sci.*, vol. 83, pp. 5939–5942, Aug. 1986, "A Large Region (≈95 kDa) of Human Factor VIII is Dispensable for in vitro Procoagulant Activity".

Kaufman et al, *Proteases in Biological Control and Biotechnol.*, J. Gen. Biochem. Supp., 10D 275 (1968).

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[57] **ABSTRACT**

Novel procoagulant proteins are disclosed which comprise the amino acid sequence:

A-X-B

wherein region A represents the polypeptide sequence Ala-20 through Arg-759 substantially as shown in Table 1; region B represents the polypeptide sequence Ser-1709 through Tyr-2351 substantially as shown in Table 1; and region X represents a polypeptide sequence comprising up to 949 amino acids substantially duplicative of sequences of amino acids within the sequence SER-760 through Arg-1708 of Table 1, wherein the amino terminus of X is covalently bonded through a peptide bond designated "-" to the carboxy terminus of A, and the carboxy terminus of X is likewise bonded to the amino terminus of B. Methods of making such proteins and their use in pharmaceutical preparations is also disclosed.

12 Claims, No Drawings

NOVEL PROCOAGULANT PROTEINS

This application is a continuation in part of U.S. Ser. No. 725,350 (filed Apr. 12, 1985), now abandoned, the contents of which are hereby incorporated by reference.

This invention relates to a novel series of proteins which exhibit procoagulant properties. These proteins have marked structural differences from human factor VIII:C, but have similar procoagulant activity.

Factor VIII:C is the blood plasma protein that is defective or absent in Hemophilia A disease. This disease is a hereditary bleeding disorder affecting approximately one in 20,000 males. The structure of factor VIII:C is described in U.S. Patent Applications Ser. Nos. 546,650 filed Oct. 28, 1983 and 644,036 filed Aug. 24, 1984, which are incorporated herein by reference and in *Nature*. 312:306, 307, 326 and 342.

One of the problems presently encountered with the use of human factor VIII:C for treatment of hemophilia arises from its antigenicity. A significant percentage of hemophiliacs have developed an immune reaction to the factor VIII:C used for their treatment. Non-hemophiliacs can also develop or acquire hemophilia when their immune systems become sensitized to factor VIII:C and produce circulating antibodies or "inhibitors" to factor VIII:C. In either case, the effect is the neutralization of whatever factor VIII:C is present in the patient, making treatment very difficult. Until now, the method of choice for treating hemophiliacs with this problem has been to administer, in cases of severe bleeding episodes, non-human factor VIII:C, such as treated porcine factor VIII:C. See Kernoff et al., *Blood* 63:31 (1984). However, the antibodies which neutralize the clotting ability of human factor VIII:C will react to a varying extent with factor VIII:C of other species, and the porcine protein is itself antigenic, thus both the short-term and long-term effectiveness of such treatment will vary.

Additionally, patients frequently display adverse reactions to infusion with the porcine factor VIII:C. The use of porcine factor VIII:C in spite of the risks has been justified because of the lack of reliably effective alternatives. Kernoff, supra at 38. The present invention provides an alternative to the administration of porcine factor VIII:C.

This invention provides for proteins which have procoagulant activity similar to that of factor VIII:C and also have substantially lower molecular weight. These proteins are schematically depicted by formula (1) as follows:

A-X-B (1)

wherein A represents a polypeptide sequence substantially duplicative of the sequence Ala-20 through Arg-759; B represents a polypeptide sequence substantially duplicative of the sequence Ser-1709 through the C-terminal Tyr-2351; and X represents a polypeptide sequence of up to 949 amino acids substantially duplicative of sequences of amino acids within the sequence Ser-760 through Arg-1708. The amino terminus of region X is covalently bonded through a peptide bond (designated "-" in formula 1) to the carboxy terminus of A. The carboxy terminus of region X is likewise bonded to the amino terminus of B. Numbering of amino acids throughout this disclosure is with reference to the numbering of amino acids in Table 1 in which the first amino acid, Met, of the leader sequence is assigned Number 1. Protein domain X may comprise a continuous but shorter sequence selected from the region Ser-760 through Arg-1708. Alternatively X may comprise two or more amino acid sequences selected from that region which are covalently bonded by a peptide bond (maintaining an ascending numerical order of amino acids).

TABLE 1

5' GAATTCCCCACTGGGTAAGTTCCTTAAAGCTCTGGAAAGAAATTCCGACTTTTCATTAAATCAGAAATT

TTACTTTTTTCCCCTCCTGGGACCTAAAGATATTTTAGAGAAGAATTAACCTTTTGCTTCTCCAGTTGAACATTTCTAGCAATAAGTC

MET	Gln	Ile	Glu	Leu	Ser	Thr	Cys	Phe	Phe	Leu	Cys	Leu	Leu	Arg	Phe	Cys	Phe	18
ATG	CAA	ATA	CAG	CTC	TCC	ACC	TGC	TTC	TTT	CTG	TGC	CTT	TTG	CCA	TTC	TCC	TTT	
Ser	Ala	Thr	Arg	Arg	Tyr	Tyr	Leu	Gly	Ala	Val	Glu	Leu	Ser	Trp	Asp	Tyr	MET	36
ACT	CCC	ACC	AGA	AGA	TAC	TAC	CTG	GGT	CCA	CTG	GAA	CTC	TCA	TGC	GAC	TAT	ATC	
Gln	Ser	Asp	Leu	Gly	Glu	Leu	Pro	Val	Asp	Ala	Arg	Phe	Pro	Pro	Arg	Val	Pro	54
CAA	AGT	GAT	CTC	GGT	CAG	CTG	CCT	GTG	CAC	GCA	AGA	TTT	CCT	CCT	AGA	CTG	CCA	
Lys	Ser	Phe	Pro	Phe	Asn	Thr	Ser	Val	Val	Tyr	Lys	Lys	Thr	Leu	Phe	Val	Glu	72
AAA	TCT	TTT	CCA	TTC	AAC	ACC	TCA	CTC	GTG	TAC	AAA	AAG	ACT	CTG	TTT	GTA	GAA	
Phe	Thr	Val	His	Leu	Phe	Asn	Ile	Ala	Lys	Pro	Arg	Pro	Pro	Trp	MET	Gly	Leu	90
TTC	ACG	GTT	CAC	CTT	TTC	AAC	ATC	CCT	AAG	CCA	ACC	CCA	CCC	TGG	ATG	CCT	CTG	
Leu	Gly	Pro	Thr	Ile	Gln	Ala	Glu	Val	Tyr	Asp	Thr	Val	Val	Ile	Thr	Leu	Lys	108
CTA	GGT	CCT	ACC	ATC	CAG	CCT	GAG	GTT	TAT	CAT	ACA	GTG	GTC	ATT	ACA	CTT	AAG	
Asn	MET	Ala	Ser	His	Pro	Val	Ser	Leu	His	Ala	Val	Gly	Val	Ser	Tyr	Trp	Lys	126
AAC	ATG	GCT	TCC	CAT	CCT	GTC	ACT	CTT	CAT	GCT	GTT	CGT	GTA	TCC	TAC	TGG	AAA	
Ala	Ser	Glu	Gly	Ala	Glu	Tyr	Asp	Asp	Gln	Thr	Ser	Gln	Arg	Glu	Lys	Glu	Asp	144
GCT	TCT	CAG	CCA	CCT	CAA	TAT	CAT	CAT	CAC	ACC	ACT	CAA	AGG	CAG	AAA	GAA	CAT	
Asp	Lys	Val	Phe	Pro	Gly	Gly	Ser	His	Thr	Tyr	Val	Trp	Gln	Val	Leu	Lys	Glu	162
GAT	AAA	GTC	TTC	CCT	CCT	GGA	ACC	CAT	ACA	TAT	CTC	TCC	CAG	CTC	CTG	AAA	GAG	
Asn	Gly	Pro	MET	Ala	Ser	Asp	Pro	Leu	cys	Leu	Thr	Tyr	Ser	Tyr	Leu	Ser	His	180
AAT	GGT	CCA	ATG	GCC	TCT	CAC	CCA	CTG	TCC	CTT	ACC	TAC	TCA	TAT	CTT	TCT	CAT	
Val	Asp	Leu	Val	Lys	Asp	Leu	Asn	Ser	Gly	Leu	Ile	Gly	Ala	Leu	Leu	Val	Cys	198
GTG	GAC	CTG	CTA	AAA	GAC	TTG	AAT	TCA	CGC	CTC	ATT	GGA	CCC	CTA	CTA	CTA	TGT	
Arg	Glu	Gly	Ser	Leu	Ala	Lys	Glu	Lys	Thr	Gln	Thr	Leu	His	Lys	Phe	Ile	Leu	216
AGA	CAA	GGG	AGT	CTG	GCC	AAG	CAA	AAG	ACA	CAG	ACC	TTG	CAC	AAA	TTT	ATA	CTA	
Leu	Phe	Ala	Val	Phe	Asp	Glu	Gly	Lys	Ser	Trp	His	Ser	Glu	Thr	Lys	Asn	Ser	234
CTT	TTT	GCT	GTA	TTT	CAT	GAA	GGG	AAA	ACT	TGC	CAC	TCA	GAA	ACA	AAG	AAC	TCC	
Leu	MET	Gln	Asp	Arg	Asp	Ala	Ala	Ser	Ala	Arg	Ala	Trp	Pro	Lys	MET	His	Thr	252
TTG	ATG	CAG	CAT	AGG	CAT	GCT	CCA	TCT	CCT	CCG	GCC	TGG	CCT	AAA	ATG	CAC	ACA	
Val	Asn	Gly	Tyr	Val	Asn	Arg	Ser	Leu	Pro	Gly	Leu	Ile	Gly	Cys	His	Arg	Lys	270
GTC	AAT	CGT	TAT	GTA	AAC	AGG	TCT	CTG	CCA	CCT	CTG	ATT	CCA	TCC	CAC	ACG	AAA	
Ser	Val	Tyr	Trp	His	Val	Ile	Gly	MET	Gly	Thr	Thr	Pro	Glu	Val	His	Ser	Ile	288
TCA	CTC	TAT	TGG	CAT	CTG	ATT	GCA	ATG	GGC	ACC	ACT	CCT	GAA	GTC	CAC	TCA	ATA	
Phe	Lue	Glu	Gly	His	Thr	Phe	leu	Val	Arg	Asn	His	Arg	Gln	Ala	Ser	Leu	Glu	306
TTC	CTC	CAA	CCT	CAC	ACA	TTT	CTT	CTC	ACC	AAC	CAT	CCC	CAC	CCG	TCC	TTG	CAA	

TABLE 1-continued

Ile	Ser	Pro	Ile	Thr	Phe	Leu	Thr	Ala	Gln	Thr	Leu	Leu	MET	Asp	Leu	Gly	Gln	324
ATC	TCG	CCA	ATA	ACT	TTC	CTT	ACT	CCT	CAA	ACA	CTC	TTG	ATG	CAC	CTT	GGA	CAG	
Phe	Leu	Leu	Phe	Cys	His	Ile	Ser	Ser	His	Gln	His	Asp	Gly	MET	Glu	Ala	Tyr	342
TTT	CTA	CTG	TTT	TCT	CAT	ATC	TCT	TCC	CAC	CAA	CAT	GAT	GGC	ATG	CAA	GCT	TAT	
Val	Lys	Val	Asp	Ser	Cys	Pro	Glu	Glu	Pro	Gln	Leu	Arg	MET	Lys	Asn	Asn	Glu	360
GTC	AAA	GTA	GAC	AGC	TGT	CCA	GAG	GAA	CCC	CAA	CTA	CGA	ATG	AAA	AAT	AAT	GAA	
Glu	Ala	Glu	Asp	Tyr	Asp	Asp	Asp	Leu	Thr	Asp	Ser	Glu	MET	Asp	Val	Val	Arg	378
GAA	GCG	GAA	GAC	TAT	GAT	GAT	GAT	CTT	ACT	CAT	TCT	GAA	ATG	GAT	CTG	GTC	AGG	
Phe	Asp	Asp	Asp	Asn	Ser	Pro	Ser	Phe	Ile	Gln	Ile	Arg	Ser	Val	Ala	Lys	Lys	396
TTT	GAT	GAT	GAC	AAC	TCT	CCT	TCC	TTT	ATC	CAA	ATT	CCC	TCA	GTT	GCC	AAG	AAG	
His	Pro	Lys	Thr	Trp	Val	His	Tyr	Ile	Aln	Ala	Glu	Glu	Glu	Asp	Trp	Asp	Tyr	414
CAT	CCT	AAA	ACT	TGG	GTA	CAT	TAC	ATT	GCT	GCT	GAA	GAG	GAG	GAC	TGG	GAC	TAT	
Ala	Pro	Leu	Val	Leu	Ala	Pro	Asp	Asp	Arg	Ser	Tyr	Lys	Ser	Gln	Tyr	Leu	Asn	432
GCT	CCC	TTA	GTC	CTC	GCC	CCC	CAT	GAC	AGA	AGT	TAT	AAA	AGT	CAA	TAT	TTG	AAC	
Asn	Gly	Pro	Gln	Arg	Ile	Gly	Arg	Lyn	Tyr	Lys	Lys	Val	Arg	Phe	MET	Ala	Tyr	450
AAT	GCC	CCT	CAG	CGG	ATT	GGT	AGG	AAC	TAC	AAA	AAA	CTC	CGA	TTT	ATG	CCA	TAC	
Thr	Asp	Glu	Thr	Phe	Lys	Thr	Arg	Glu	Ala	Ile	Gln	His	Glu	Ser	Gly	Ile	Leu	468
ACA	CAT	GAA	ACC	TTT	AAG	ACT	CGT	GAA	CCT	ATT	CAG	CAT	GAA	TCA	CCA	ATC	TTG	
Gly	Pro	Leu	Leu	Tyr	Gly	Glu	Val	Gly	Asp	Thr	Leu	Leu	Ile	Ile	Phe	Lys	Asn	486
GGA	CCT	TTA	CTT	TAT	GGG	GAA	GTT	CCA	GAC	ACA	CTG	TTC	ATT	ATA	TTT	AAG	AAT	
Gln	Ala	Ser	Arg	Pro	Tyr	Asn	Ile	Tyr	Pro	His	Gly	Ile	Thr	Asp	Val	Arg	Pro	504
CAA	GCA	AGC	ACA	CCA	TAT	AAC	ATC	TAC	CCT	CAC	CGA	ATC	ACT	CAT	GTC	CGT	CCT	
Leu	Tyr	Ser	Arg	Arg	Leu	Pro	Lys	Gly	Val	Lys	His	Leu	Lys	Asp	Phe	Pro	Ile	522
TTG	TAT	TCA	AGG	AGA	TTA	CCA	AAA	GGT	GTA	AAA	CAT	TTG	AAG	GAT	TTT	CCA	ATT	
Leu	Pro	Gly	Glu	Ile	Phe	Lys	Tyr	Lys	Trp	Thr	Val	Thr	Val	Glu	Asp	Gly	Pro	540
CTG	CCA	GGA	GAA	ATA	TTC	AAA	TAT	AAA	TGG	ACA	GTG	ACT	GTA	CAA	CAT	GGG	CCA	
Thr	Lys	Ser	Asp	Pro	Arg	Cys	Leu	Thr	Arg	Tyr	Tyr	Ser	Ser	Phe	Val	Asn	MET	558
ACT	AAA	TCA	GAT	CCT	CGG	TGC	CTG	ACC	CGC	TAT	TAC	TCT	ACT	TTC	GTT	AAT	ATG	
Glu	Arg	Asp	Leu	Ala	Ser	Gly	Leu	Ile	Gly	Pro	Leu	Leu	Ile	Cys	Tyr	Lys	Glu	576
GAG	AGA	GAT	CTA	GCT	TCA	GGA	CTC	ATT	CGC	CCT	CTC	CTC	ATC	TGC	TAC	AAA	GAA	
Ser	Val	Asp	Gln	Arg	Gly	Asn	Gln	Ile	MET	Ser	Asp	Lys	Arg	Asn	Val	Ile	Leu	594
TCT	GTA	GAT	CAA	AGA	GGA	AAC	CAG	ATA	ATG	TCA	GAC	AAG	AGG	AAT	GTC	ATG	CTG	
Phe	Ser	Vnl	Phe	Asp	Glu	Asn	Arg	Ser	Trp	Tyr	Leu	Thr	Glu	Asp	Ile	Gln	Arg	612
TTT	TCT	GTA	TTT	CAT	CAG	AAC	CGA	AGC	TCG	TAC	CTC	ACA	CAG	AAT	ATA	CAA	CGC	
Phe	Leu	Pro	Asn	Pro	Ala	Gly	Val	Gln	Leu	Glu	Asp	Pro	Glu	Phe	Gln	Ala	Ser	630
TTT	CTC	CCC	AAT	CCA	CCT	CGA	GTG	CAG	CTT	CAG	CAT	CCA	GAG	TTC	CAA	GCC	TCC	

TABLE 1-continued

Asn AAC	Ile ATC	MET ATG	His CAC	Ser ACC	Ile ATC	Asn AAT	Gly GGC	Tyr TAT	Val CTT	Phe TTT	Asp CAT	Ser ACT	Leu TTG	Gln CAG	Leu TTG	Ser TCA	Val GTT	648
Cys TGT	Leu TTG	His CAT	Glu CAG	Val CTG	Ala CCA	Tyr TAC	Trp TGG	Tyr TAC	Ile ATT	Leu CTA	Ser ACC	Ile ATT	Gly GGA	Ala GCA	Gln CAG	Thr ACT	Asp CAC	666
Phe TTC	Leu CTT	Ser TCT	Val GTC	Phe TTC	Phe TTC	Ser TCT	Gly GGA	Tyr TAT	Thr ACC	Phe TTC	Lys AAA	His CAC	Lys AAA	MET ATG	Val GTC	Tyr TAT	Glu CAA	684
Asp GAC	Thr ACA	Leu CTC	Thr ACC	Leu CTA	Phe TTC	Pro CCA	Phe TTC	Ser TCA	Gly GGA	Glu CAA	Thr ACT	Val GTC	Phe TTC	MET ATG	Ser TCG	MET ATG	Glu GAA	702
Asn AAC	Pro CCA	Gly GGT	Leu CTA	Trp TGG	Ile ATT	Leu CTG	Gly GGG	Cys TGC	His CAC	Asn AAC	Ser TCA	Asp GAC	Phe TTT	Arg CGG	Asn AAC	Arg AGA	Gly CCC	720
MET ATG	Thr ACC	Ala CCC	Leu TTA	Leu CTG	Lys AAG	Val GTT	Ser TCT	Ser AGT	Cys TGT	Asp GAC	Lys AAG	Asn AAC	Thr ACT	Gly GGT	Asp GAT	Tyr TAT	Tyr TAC	738
Glu GAG	Asp GAC	Ser ACT	Tyr TAT	Glu GAA	Asp CAT	Ile ATT	Ser TCA	Ala GCA	Tyr TAC	Leu TTG	Leu CTG	Ser ACT	Lys AAA	Asn AAC	Asn AAT	Ala GCC	Ile ATT	756
Glu GAA	Pro CCA	Arg AGA	Ser ACC	Phe TTC	Ser TCC	Gln CAG	Asn AAT	Ser TCA	Arg AGA	His CAC	Pro CCT	Ser AGC	Thr ACT	Arg AGG	Gln CAA	Lys AAG	Gln CAA	774
Phe TTT	Asn AAT	Ala GCC	Thr ACC	Thr ACA	Ile ATT	Pro CCA	Glu CAA	Asn AAT	Asp GAC	Ile ATA	Glu CAG	Lys AAG	Thr ACT	Asp CAC	Pro CCT	Trp TGG	Phe TTT	792
Ala GCA	His CAC	Arg AGA	Thr ACA	Pro CCT	MET ATG	Pro CCT	Lys AAA	Ile ATA	Gln CAA	Asn AAT	Val GTC	Ser TCC	Ser TCT	Ser ACT	Asp GAT	Leu TTG	Leu TTG	810
MET ATG	Leu CTC	Leu TTG	Arg CGA	Gln CAG	Ser ACT	Pro CCT	Thr ACT	Pro CCA	His CAT	Gly GGG	Leu CTA	Ser TCC	Leu TTA	Ser TCT	Asp GAT	Leu CTC	Gln CAA	828
Glu GAA	Ala GCC	Lys AAA	Tyr TAT	Glu GAG	Thr ACT	Phe TTT	Ser TCT	Asp GAT	Asp GAT	Pro CCA	Ser TCA	Pro CCT	Gly GGA	Ala GCA	Ile ATA	Asp CAC	Ser ACT	846
Asn AAT	Asn AAC	Ser AGC	Leu CTG	Ser TCT	Glu GAA	MET ATG	Thr ACA	His CAC	Phe TTC	Arg ACC	Pro CCA	Gln CAG	Leu CTC	His CAT	His CAC	Ser ACT	Gly GGG	864
Asp GAC	MET ATG	Val GTA	Phe TTT	Thr ACC	Pro CCT	Glu GAG	Ser TCA	Gly GGC	Leu CTC	Gln CAA	Leu TTA	Arg AGA	Leu TTA	Asn AAT	Glu CAG	Lys AAA	Leu CTG	882
Gly GGG	Thr ACA	Thr ACT	Ala GCA	Ala GCA	Thr ACA	Glu GAG	Leu TTC	Lys AAG	Lys AAA	Leu CTT	Asp CAT	Phe TTC	Lys AAA	Val CTT	Ser TCT	Ser ACT	Thr ACA	900
Ser TCA	Asn AAT	Asn AAT	Leu CTG	Ile ATT	Ser TCA	Thr ACA	Ile ATT	Pro CCA	Ser TCA	Asp GAC	Asn AAT	Leu TTG	Ala GCA	Ala GCA	Gly CCT	Thr ACT	Asp CAT	918
Asn AAT	Thr ACA	Ser AGT	Ser TCC	Leu TTA	Gly GGA	Pro CCC	Pro CCA	Ser ACT	MET ATG	Pro CCA	Val GTT	His CAT	Tyr TAT	Asp CAT	Ser ACT	Gln CAA	Leu TTA	936
Asp	Thr	Thr	Leu	Phe	Gly	Lys	Lys	Ser	Ser	Pro	Leu	Thr	Glu	Ser	Gly	Gly	Pro	954

TABLE 1-continued

CAT	ACC	ACT	CTA	TTT	GGC	AAA	AAG	TCA	TCT	CCC	CTT	ACT	GAG	TCT	GGT	GCA	CCT	
Leu	Ser	Leu	Ser	Glu	Glu	Asn	Asn	Asp	Ser	Lys	Leu	Leu	Glu	Ser	Gly	Leu	MET	972
CTG	AGC	TTG	ACT	CAA	CAA	AAT	AAT	CAT	TCA	AAG	TTG	TTA	CAA	TCA	CCT	TTA	ATC	
Asn	Ser	Gln	Glu	Ser	Ser	Trp	Gly	Lys	Asn	Val	Ser	Ser	Thr	Glu	Ser	Gly	Arg	990
AAT	ACC	CAA	CAA	ACT	TCA	TGG	CGA	AAA	AAT	CTA	TCG	TCA	ACA	CAG	ACT	CGT	ACC	
Leu	Phe	Lys	Gly	Lys	Arg	Ala	His	Gly	Pro	Ala	Leu	Leu	Thr	Lys	Asp	Asn	Ala	1,008
TTA	TTT	AAA	CGG	AAA	AGA	GCT	CAT	GCA	CCT	CCT	TTG	TTG	ACT	AAA	CAT	AAT	GCC	
Leu	Phe	Lys	Val	Ser	Ile	Ser	Leu	Leu	Lys	Thr	Asn	Lys	Thr	Ser	Asn	Asn	Ser	1,026
TTA	TTC	AAA	GTT	AGC	ATC	TCT	TTG	TTA	AAG	ACA	AAC	AAA	ACT	TCC	AAT	AAT	TCA	
Ala	Thr	Asn	Arg	Lys	Thr	His	Ile	Asp	Gly	Pro	Ser	Leu	Leu	Ile	Glu	Asn	Ser	1,044
CCA	ACT	AAT	ACA	AAG	ACT	CAC	ATT	CAT	CGC	CCA	TCA	TTA	TTA	ATT	GAG	AAT	AGT	
Pro	Ser	Val	Trp	Gln	Asn	Ile	Leu	Glu	Ser	Asp	Thr	Glu	Phe	Lys	Lys	Val	Thr	1,062
CCA	TCA	GTC	TGG	CAA	AAT	ATA	TTA	GAA	AGT	GAC	ACT	CAG	TTT	AAA	AAA	GTG	ACA	
Pro	Leu	Ile	His	Asp	Arg	MET	Leu	MET	Asp	Lys	Asn	Ala	Thr	Ala	Leu	Arg	Leu	1,080
CCT	TTG	ATT	CAT	GAC	AGA	ATG	CTT	ATG	GAC	AAA	AAT	GCT	ACA	CCT	TTC	AGG	CTA	
Asn	His	MET	Ser	Asn	Lys	Thr	Thr	Ser	Ser	Lys	Asn	MET	Glu	MET	Val	Gln	Gln	1,098
AAT	CAT	ATG	TCA	AAT	AAA	ACT	ACT	TCA	TCA	AAA	ACC	ATG	GAA	ATG	CTC	CAA	CAG	
Lys	Lys	Glu	Gly	Pro	Ile	Pro	Pro	Asp	Ala	Gln	Asn	Pro	Asp	MET	Ser	Phe	Phe	1,116
AAA	AAA	GAG	GGC	CCC	ATT	CCA	CCA	GAT	CCA	CAA	AAT	CCA	GAT	ATG	TCG	TTC	TTT	
Lys	MET	Leu	Phe	Leu	Pro	Glu	Ser	Ala	Arg	Trp	Ile	Gln	Arg	Thr	His	Gly	Lys	1,134
AAG	ATG	CTA	TTC	TTG	CCA	GAA	TCA	CCA	ACC	TGG	ATA	CAA	AGG	ACT	CAT	GCA	AAG	
Asn	Ser	Leu	Asn	Ser	Gly	Gln	Gly	Pro	Ser	Pro	Lys	Gln	Leu	Val	Ser	Leu	Gly	1,152
AAC	TCT	CTG	AAC	TCT	GGG	CAA	GGC	CCC	ACT	CCA	AAC	CAA	TTA	GTA	TCC	TTA	GCA	
Pro	Glu	Lys	Ser	Val	Glu	Gly	Gln	Asn	Phe	Leu	Ser	Glu	Lys	Asn	Lys	Val	Val	1,170
CCA	GAA	AAA	TCT	GTG	GAA	GGT	CAG	AAT	TTC	TTG	TCT	GAG	AAA	AAC	AAA	GTG	GTA	
Val	Gly	Lys	Gly	Gly	Phe	Thr	Lys	Asp	Val	Gly	Leu	Lys	Glu	MET	Val	Phe	Pro	1,188
GTA	GGA	AAG	GGT	GAA	TTT	ACA	AAG	CAC	GTA	CGA	CTC	AAA	GAG	ATG	CTT	TTT	CCA	
Ser	Ser	Arg	Asn	Leu	Phe	Leu	Thr	Asn	Leu	Asp	Asn	Leu	His	Glu	Asn	Asn	Thr	1,206
AGC	AGC	ACA	AAC	CTA	TTT	CTT	ACT	AAC	TTG	GAT	AAT	TTA	CAT	GAA	AAT	AAT	ACA	
His	Asn	Gln	Glu	Lys	Lys	Ile	Gln	Glu	Glu	Ile	Glu	Lys	Lys	Glu	Thr	Leu	Ile	1,224
CAC	AAT	CAA	CAA	AAA	AAA	ATT	CAG	CAA	CAA	ATA	GAA	AAG	AAG	GAA	ACA	TTA	ATC	
Gln	Glu	Asn	Val	Val	Leu	Pro	Gln	Ile	His	Thr	Val	Thr	Gly	Thr	Lys	Asn	Phe	1,242
CAA	GAG	AAT	GTA	GTT	TTG	CCT	CAG	ATA	CAT	ACA	CTG	ACT	GGC	ACT	AAG	AAT	TTC	
MET	Lys	Asn	Leu	Phe	Leu	Leu	Ser	Thr	Arg	Gln	Asn	Val	Glu	Gly	Ser	Tyr	Glu	1,260
ATG	AAC	AAC	CTT	TTC	TAA	CTG	ACC	ACT	AGG	GAA	AAT	GTA	GAA	GGT	TCA	TAT	GAG	

TABLE 1-continued

Gly GGG	Ala GCA	Tyr TAT	Ala CCT	Pro CCA	Val GTA	Leu CTT	Gln CAA	Asp CAT	Phe TTT	Arg AGG	Ser TCA	Leu TTA	Asn AAT	Asp GAT	Ser TCA	Thr ACA	Asn AAT	1,278
Arg AGA	Thr ACA	Lys AAG	Lys AAA	His CAC	Thr ACA	Ala CCT	His CAT	Phe TTC	Ser TCA	Lys AAA	Lys AAA	Gly CGG	Glu CAG	Glu CAA	Glu CAA	Asn AAC	Leu TTG	1,296
Glu CAA	Gly GGC	Leu TTG	Gly GGA	Asn AAT	Gln CAA	Thr ACC	Lys AAG	Gln CAA	Ile ATT	Val CTA	Glu CAG	Lys AAA	Tyr TAT	Ala CCA	Cys TGC	Thr ACC	Thr ACA	1,314
Arg AGC	Ile ATA	Ser TCT	Pro CCT	Asn AAT	Thr ACA	Ser AGC	Gln CAG	Gln CAG	Asn AAT	Phe TTT	Val CTC	Thr ACG	Gln CAA	Arg CCT	Ser ACT	Lys AAG	Arg AGA	1,332
Ala CCT	Leu TTG	Lys AAA	Gln CAA	Phe TTC	Arg AGA	Leu CTC	Pro CCA	Leu CTA	Glu GAA	Glu GAA	Thr ACA	Glu CAA	Leu CTT	Glu GAA	Lys AAA	Arg AGG	Ile ATA	1,350
Ile ATT	Val GTG	Asp GAT	Asp GAC	Thr ACC	Ser TCA	Thr ACC	Gln CAC	Trp TGG	Ser TCC	Lys AAA	Asn AAC	MET ATG	Lys AAA	His CAT	Leu TTC	Thr ACC	Pro CCG	1,368
Ser AGC	Thr ACC	Leu CTC	Thr ACA	Gln CAG	Ile ATA	Asp GAC	Tyr TAC	Asn AAT	Glu GAG	Lys AAG	Glu GAC	Lys AAA	Gly GGG	Alu GCC	Ile ATT	Thr ACT	Cln CAG	1,386
Ser TCT	Pro CCC	Leu TTA	Ser TCA	Asp GAT	Cys TGC	Leu CTT	Thr ACG	Arg AGG	Ser ACT	His CAT	Ser AGC	Ile ATC	Pro CCT	Gln CAA	Ala GCA	Asn AAT	Arg AGA	1,404
Ser TCT	Pro CCA	Leu TTA	Pro CCC	Ile ATT	Ala GCA	Lys AAG	Val GTA	Ser TCA	Ser TCA	Phe TTT	Pro CCA	Ser TCT	Ile ATT	Arg AGA	Pro CCT	Ile ATA	Tyr TAT	1,422
Leu CTG	Thr ACC	Arg AGG	Val GTC	Leu CTA	Phe TTC	Gln CAA	Asp GAC	Asn AAC	Ser TCT	Ser TCT	His CAT	Leu CTT	Pro CCA	Ala GCA	Ala GCA	Ser TCT	Tyr TAT	1,440
Arg ACA	Lys AAG	Lys AAA	Asp GAT	Ser TCT	Gly GGG	Val GTC	Gln CAA	Glu GAA	Ser AGC	Ser ACT	His CAT	Phe TTC	Leu TTA	Gln CAA	Gly CGA	Ala CCC	Lys AAA	1,458
Lys AAA	Asn AAT	Asn AAC	Leu CTT	Ser TCT	Leu TTA	Ala GCC	Ile ATT	Leu CTA	Thr ACC	Leu TTG	Glu GAG	MET ATG	Thr ACT	Gly CGT	Asp CAT	Gln CAA	Arg AGA	1,476
Glu GAG	Val GTT	Gly GGC	Ser TCC	Leu CTG	Gly GGG	Thr ACA	Ser ACT	Ala GCC	Thr ACA	Asn AAT	Ser TCA	Val GTC	Thr ACA	Tyr TAC	Lys AAC	Lys AAA	Val GTT	1,494
Glu CAG	Asn AAC	Thr ACT	Val GTT	Leu CTC	Pro CCG	Lys AAA	Pro CCA	Asp GAC	Leu TTG	Pro CCC	Lys AAA	Thr ACA	Ser TCT	Gly CGC	Lys AAA	Val CTT	Glu GAA	1,512
Leu TTG	Leu CTT	Pro CCA	Lys AAA	Val GTT	His CAC	Ile ATT	Tyr TAT	Gln CAG	Lys AAG	Asp CAC	Leu CTA	Phe TTC	Pro CCT	Thr ACG	Glu GAA	Thr ACT	Ser AGC	1,530
Asn AAT	Gly CCG	Ser TCT	Pro CCT	Gly GGC	His CAT	Leu CTG	Asp GAT	Leu CTC	Val GTG	Glu CAA	Gly GGG	Ser AGC	Leu CTT	Leu CTT	Gln CAG	Gly CGA	Thr ACA	1,548
Glu GAG	Gly GGA	Ala GCG	Ile ATT	Lys AAC	Trp TGG	Asn AAT	Glu GAA	Ala CCA	Asn AAC	Arg AGA	Pro CCT	Gly CGA	Lys AAA	Val CTT	Pro CCC	Phe TTT	Leu CTG	1,566
Arg AGA	Val GTA	Ala GCA	Thr ACA	Glu GAA	Ser AGC	Ser TCT	Ala GCA	Lys AAG	Thr ACT	Pro CCC	Ser TCC	Lys AAC	Leu CTA	Leu TTG	Asp CAT	Pro CCT	Leu CTT	1,584

TABLE 1-continued

Ala GCT	Trp TGG	Asp GAT	Asn AAC	His CAC	Tyr TAT	Gly GGT	Thr ACT	Gln CAG	Ile ATA	Pro CCA	Lys AAA	Glu GAA	Glu GAG	Trp TGG	Lys AAA	Ser TCC	Gln CAA	1,602
Glu GAG	Lys AAG	Ser TCA	Pro CCA	Glu GAA	Lys AAA	Thr ACA	Ala GCT	Phe TTT	Lys AAG	Lys AAA	Lys AAG	Asp GAT	Thr ACC	Ile ATT	Leu TTG	Ser TCC	Leu CTG	1,520
Asn AAC	Ala GCT	Cys TGT	Glu GAA	Ser AGC	Asn AAT	His CAT	Ala GCA	Ile ATA	Ala GCA	Ala CCA	Ile ATA	Asn AAT	Glu GAG	Gly CGA	Gln CAA	Asn AAT	Lys AAG	1,638
Pro CCC	Glu GAA	Ile ATA	Glu GAA	Val GTC	Thr ACC	Trp TCC	Ala GCA	Lys AAG	Gln CAA	Gly CGT	Arg AGG	Thr ACT	Glu CAA	Arg AGG	Leu CTC	Cys TCC	Ser TCT	1,656
Gln CAA	Asn AAC	Pro CCA	Pro CCA	Val CTC	Leu TTC	Lys AAA	Arg CCC	His CAT	Gln CAA	Arg CGC	Glu CAA	Ile ATA	Thr ACT	Arg CGT	Thr ACT	Thr ACT	Leu CTT	1,674
Gln CAG	Ser TCA	Asp GAT	Gln CAA	Glu GAG	Glu GAA	Ile ATT	Asp CAC	Tyr TAT	Asp CAT	Asp CAT	Thr ACC	Ile ATA	Ser TCA	Val GTT	Glu GAA	MET ATG	Lys AAG	1,692
Lys AAG	Glu GAA	Asp GAT	The TTT	Asp GAC	Ile ATT	Tyr TAT	Asp CAT	Glu GAG	Asp CAT	Glu GAA	Asn AAT	Gln CAC	Ser AGC	Pro CCC	Arg CGC	Ser AGC	Phe TTT	1,710
Gln CAA	Lys AAG	Lys AAA	Thr ACA	Arg CCA	His CAC	Tyr TAT	Phe TTT	Ile ATT	Ala GCT	Ala GCA	Val GTG	Glu GAG	Arg AGC	Leu CTC	Trp TGG	Asp GAT	Tyr TAT	1,728
Gly CCG	MET ATG	Ser AGT	Ser AGC	Ser TCC	Pro CCA	His CAT	Val GTT	Leu CTA	Arg AGA	Asn AAC	Arg AGG	Ala GCT	Gln CAG	Ser AGT	Gly GGC	Ser ACT	Val GTC	1,746
Pro CCT	Gln CAG	Phe TTC	Lys AAG	Lys AAA	Val GTT	Val GTT	Phe TTC	Gln CAG	Glu CAA	Phe TTT	Thr ACT	Asp GAT	Gly CGC	Ser TCC	Phe TTT	Thr ACT	Gln CAG	1,764
Pro CCC	Leu TTA	Tyr TAC	Arg CCT	Gly GGA	Glu GAA	Leu CTA	Asn AAT	Glu GAA	His CAT	Leu TTG	Gly GGA	Leu CTC	Leu CTG	Gly GGG	Pro CCA	Tyr TAT	Ile ATA	1,782
Arg AGA	Ala GCA	Glu GAA	Val GTT	Glu GAA	Asp GAT	Asn AAT	Ile ATC	MET ATG	Val GTA	Thr ACT	Phe TTC	Arg AGA	Asn AAT	Gln CAG	Ala GCC	Ser TCT	Arg CGT	1,800
Pro CCC	Tyr TAT	Ser TCC	Phe TTC	Tyr TAT	Ser TCT	Ser AGC	Leu CTT	Ile ATT	Ser TCT	Tyr TAT	Glu GAG	Glu GAA	Asp GAT	Gln CAG	Arg AGG	Gln CAA	Gly GGA	1,818
Ala GCA	Glu GAA	Pro CCT	Arg AGA	Lys AAA	Asn AAC	Phe TTT	Val GTC	Lys AAG	Pro CCT	Asn AAT	Glu GAA	Thr ACC	Lys AAA	Thr ACT	Tyr TAC	Phe TTT	Trp TGG	1,836
Lys AAA	Val CTG	Gln CAA	His CAT	His CAT	MET ATG	Ala GCA	Pro CCC	Thr ACT	Lys AAA	Asp GAT	Glu GAG	Phe TTT	Asp GAC	Cys TGC	Lys AAA	Ala GCC	Trp TGG	1,854
Ala GCT	Tyr TAT	Phe TTC	Ser TCT	Asp GAT	Val GTT	Asp GAC	Leu CTG	Glu GAA	Lys AAA	Asp CAT	Val GTG	His CAC	Ser TCA	Gly GGC	Leu CTG	Ile ATT	Gly GGA	1,872
Pro CCC	Leu CTT	Leu CTG	Val CTC	Cys TGC	His CAC	Thr ACT	Asn AAC	Thr ACA	Leu CTG	Asn AAC	Pro CCT	Ala GCT	His CAT	Gly CCG	Arg AGA	Gln CAA	Val CTG	1,890
Thr	Val	Glu	Glu	Phe	Ala	Leu	Phe	Phe	Thr	Ile	Phe	Asp	Glu	Thr	Lys	Ser	Trp	1,908

TABLE 1-continued

ACA	CTA	CAG	GAA	TTT	GCT	CTG	TTT	TTC	ACC	ATC	TTT	CAT	GAG	ACC	AAA	AGC	TCG	
Thy	Phe	Thr	Glu	Asn	MET	Glu	Arg	Asn	Cys	Arg	Ala	Pro	Cys	Asn	Ile	Gln	MET	1,926
TAC	TTC	ACT	CAA	AAT	ATG	CAA	AGA	AAC	TGC	ACC	CCT	CCC	TGC	AAT	ATC	CAG	ATG	
Glu	Asp	Pro	Thr	Phe	Lys	Glu	Asn	Thr	Arg	Phe	His	Ala	Ile	Asn	Gly	Tyr	Ile	1,944
CAA	GAT	CCC	ACT	TTT	AAA	CAG	AAT	TAT	CGC	TTC	CAT	GCA	ATC	AAT	CGC	TAC	ATA	
MET	Asp	Thr	Leu	Pro	Gly	Leu	Val	MET	Ala	Gln	Asp	Gln	Arg	Ile	Arg	Trp	Tyr	1,962
ATG	CAT	ACA	CTA	CCT	GGC	TTA	GTA	ATG	GCT	CAG	GAT	CAA	AGG	ATT	CGA	TCG	TAT	
Leu	Leu	Ser	MET	Gly	Ser	Asn	Glu	Asn	Ile	His	Ser	Ile	His	Phe	Ser	Gly	His	1,980
CTC	CTC	AGC	ATG	CGC	AGC	AAT	CAA	AAC	ATC	CAT	TCT	ATT	CAT	TTC	ACT	CCA	CAT	
Val	Phe	Thr	Val	Arg	Lys	Lys	Glu	Glu	Tyr	Lys	MET	Ala	Leu	Tyr	Asn	Leu	Tyr	1,998
GTG	TTC	ACT	CTA	CCA	AAA	AAA	CAG	GAG	TAT	AAA	ATG	GCA	CTG	TAC	AAT	CTC	TAT	
Pro	Gly	Val	Phe	Glu	Thr	Val	Glu	MET	Leu	Pro	Ser	Lys	Ala	Gly	Ile	Trp	Arg	2,016
CCA	CGT	GTT	TTT	GAC	ACA	GTG	GAA	ATG	TTA	CCA	TCC	AAA	GCT	GGA	ATT	TCC	CGG	
Val	Glu	Cys	Leu	Ile	Gly	Glu	His	Leu	His	Ala	Gly	MET	Ser	Thr	Leu	Phe	Leu	2,034
GTG	GAA	TGC	CTT	ATT	CCC	GAC	CAT	CTA	CAT	CCT	CGG	ATG	AGC	ACA	CTT	TTT	CTG	
Val	Tyr	Ser	Asn	Lys	Cys	Glu	Thr	Pro	Leu	Gly	MET	Ala	Ser	Gly	His	Ile	Arg	2,052
GTG	TAC	AGC	AAT	AAG	TGT	CAG	ACT	CCC	CTG	GGA	ATG	GCT	TCT	GGA	CAC	ATT	AGA	
Asp	Phe	Gln	Ile	Thr	Ala	Ser	Gly	Gln	Tyr	Gly	Gln	Trp	Ala	Pro	Lys	Leu	Ala	2,070
CAT	TTT	CAG	ATT	ACA	GCT	TCA	CGA	CAA	TAT	GCA	CAG	TGG	GCC	CCA	AAG	CTG	GCC	
Arg	Leu	His	Tyr	Ser	Gly	Ser	Ile	Asn	Ala	Trp	Set	Thr	Lys	Glu	Pro	Phe	Ser	2,088
AGA	CTT	CAT	TAT	TCC	GGA	TCA	ATC	AAT	GCC	TGG	AGC	ACC	AAG	GAG	CCC	TTT	TCT	
Trp	Ile	Lys	Val	Asp	Leu	Leu	Ala	Pro	MET	Ile	Ile	His	Gly	Ile	Lys	Thr	Gln	2,106
TGC	ATC	AAG	GTG	CAT	CTG	TTG	GCA	CCA	ATG	ATT	ATT	CAC	GGC	ATC	AAG	ACC	CAG	
Gly	Ala	Arg	Gln	Lys	Phe	Ser	Ser	Leu	Tyr	Ile	Ser	Gln	Phe	Ile	Ile	MET	Tyr	2,124
GGT	GCC	CGT	CAG	AAG	TTC	TCC	AGC	CTC	TAC	ATC	TCT	CAG	TTT	ATC	ATC	ATG	TAT	
Ser	Leu	Asp	Gly	Lys	Lys	Trp	Gln	Thr	Tyr	Arg	Gly	Asn	Ser	Thr	Gly	Thr	Leu	2,142
AGT	CTT	GAT	GGG	AAG	AAG	TGG	CAG	ACT	TAT	CGA	GGA	AAT	TCC	ACT	CCA	ACC	TTA	
MET	Val	Phe	Phe	Gly	Asn	Val	Asp	Ser	Ser	Gly	Ile	Lys	His	Asn	Ile	Phe	Asn	2,160
ATG	GTC	TTC	TTT	GGC	AAT	CTC	CAT	TCA	TCT	CCC	ATA	AAA	CAC	AAT	ATT	TTT	AAC	
Pro	Pro	Ile	Ile	Ala	Arg	Tyr	Ile	Arg	Leu	His	Pro	Thr	His	Tyr	Ser	Ile	Arg	2,178
CCT	CCA	ATT	ATT	GCT	CGA	TAC	ATC	CGT	TTG	CAC	CCA	ACT	CAT	TAT	AGC	ATT	CCC	
Ser	Thr	Leu	Arg	MET	Glu	Leu	MET	Gly	Cys	Asp	Leu	Asn	Ser	Cys	Ser	MET	Pro	2,196
AGC	ACT	CTT	CGC	ATG	GAG	TTG	ATG	CCC	TGT	GAT	TTA	AAT	ACT	TCC	AGC	ATG	CCA	
Leu	Gly	MET	Glu	Ser	Lys	Ala	Ile	Ser	Asp	Ala	Gln	Ile	Thr	Ala	Ser	Ser	Tyr	2,214
TTG	GCA	ATG	GAG	AGT	AAA	GCA	ATA	TCA	CAT	GCA	CAG	ATT	ACT	GCT	TCA	TCC	TAC	

TABLE 1-continued

Phe	Thr	Asn	MET	Phe	Ala	Thr	Trp	Ser	Pro	Ser	Lys	Ala	Arg	Leu	His	Leu	Gln	2,232
TTT	ACC	AAT	ATG	TTT	GCC	ACC	TGG	TCT	CCT	TCA	AAA	GCT	CGA	CTT	CAC	CTC	CAA	
Gly	Arg	Ser	Asn	Ala	Trp	Arg	Pro	Gln	Val	Asn	Asn	Pro	Lys	Glu	Trp	Leu	Gly	2,250
GGG	AGG	ACT	AAT	GCC	TGG	AGA	CCT	CAG	GTG	AAT	AAT	CCA	AAA	CAG	TGG	CTG	CAA	
Val	Asp	Phe	Gln	Lys	Thr	MET	Lys	Val	Thr	Gly	Val	Thr	Thr	Gln	Gly	Val	Lys	2,268
GTG	GAC	TTC	CAG	AAG	ACA	ATG	AAA	CTC	ACA	GGA	GTA	ACT	ACT	CAG	GGA	CTA	AAA	
Ser	Leu	Leu	Thr	Ser	MET	Tyr	Val	Lys	Glu	Phe	Leu	Ile	Ser	Ser	Ser	Gln	Asp	2,286
TCT	CTG	CTT	ACC	ACC	ATG	TAT	GTG	AAG	GAG	TTC	CTC	ATC	TCC	ACC	ACT	CAA	CAT	
Gly	His	Gln	Trp	Thr	Leu	Phe	Phe	Gln	Asn	Gly	Lys	Val	Lys	Val	Phe	Gln	Gly	2,304
GGC	CAT	CAG	TGG	ACT	CTC	TTT	TTT	CAG	AAT	CCC	AAA	CTA	AAC	CTT	TTT	CAG	CCA	
Asn	Gln	Asp	Ser	Phe	Thr	Pro	Val	Val	Asn	Ser	Leu	Asp	Pro	Pro	Leu	Leu	Thr	2,322
AAT	CAA	GAC	TCC	TTC	ACA	CCT	GTG	GTG	AAC	TCT	CTA	GAC	CCA	CCG	TTA	CTG	ACT	
Arg	Tyr	Leu	Arg	Ile	His	Pro	Gln	Ser	Trp	Val	His	Gln	Ile	Ala	Leu	Arg	MET	2,340
CGC	TAC	CTT	CGA	ATT	CAC	CCC	CAG	AGT	TGG	CTC	CAC	CAG	ATT	CCC	CTG	ACC	ATG	
Glu	Val	Leu	Gly	Cys	Glu	Ala	Gln	Asp	Leu	Tyr	End							2,352
GAG	GTT	CTG	GCC	TGC	GAG	GCA	CAG	GAC	CTC	TAC	TGA	GGGTGGCCACTGCATGCCCACCTGCCACTG						

CCGTCACCTCTCCCTCCTCAGCTCCAGGGCATGTGTCCCTCCCTGGCTTGCTTCTACCTTTGTGCTAAATCCTAGCAGACACTCCCTTG

AAGCCTCCTGAATTAACTATCATGACTCCTGCATTTCTTTGGTGGGGGGCCAGGAGGCTGCATCCATTTTAACTTAACTCTTACCTATT

TTCTGCAGCTGCTCCCAGA

By way of example, one compound of this invention contains a region X comprising the amino acid sequence of Ser-760 to Pro-1000 followed by the amino acid sequence of Asp-1582 to Arg-1708. That compound thus comprises the polypeptide sequence of Ala-20 to Pro-1000 covalently linked by a peptide bond to amino acids Asp-1582 to Tyr-2351. Another exemplary compound contains a region X comprising the amino acid sequence Ser-760 to Thr-778 followed by the sequence Pro-1659 to Arg-1708. That compound thus comprises the polypeptide sequence Ala-20 to Thr-778 covalently linked by a peptide bond to the sequence Pro-1659 through Tyr-2351. Still another exemplary compound contains a region X comprising the amino acid sequence Ser-760 to Thr-778 followed by the sequence Glu-1694 to Arg-1708. That compound thus comprises the polypeptide sequence Ala-20 to Thr-778 covalently linked by a peptide bond to amino acids Glu-1694 through Tyr-2351.

These exemplary compounds are depicted schematically in Table 2.

The amino acid sequence represented by X should be selected so that it does not substantially reduce the procoagulant activity of the molecule, which activity can be conveniently assayed by conventional methods. Compound (2) of Table 2 is a presently preferred embodiment.

The procoagulant protein may be produced by appropriate host cells transformed by factor VIII:C DNA which has been specifically altered by use of any of a variety of site-specific mutagenesis techniques which will be familiar to those of ordinary skill in the art of recombinant DNA.

The starting materials may be a DNA sequence which codes for the complete factor VIII:C molecule, e.g., the complete human factor VIII:C as shown in Table 1, a truncated version of that sequence, or it may comprise segments of that DNA sequence, so long as the starting materials contain at least sufficient DNA to code for the amino acid sequences of the desired polypeptide.

TABLE 2

EXEMPLARY COMPOUNDS A-X-B

Compound	Amino Acid Sequence	X	Deletion
(human factor VIII:c)	(Ala_{20}→Tyr_{2351})	(Ser_{760}→Arg_{1708})	0
1	(Ala_{20}→Pro_{1000})—(Asp_{1582}→Tyr_{2351})	(Ser_{760}→Pro_{1000})—(Asp_{1582}→Arg_{1708})	581
2	(Ala_{20}→Thr_{778})—(Pro_{1659}→Tyr_{2351})	(Ser_{760}→Thr_{778})—(Pro_{1659}→Arg_{1708})	880
3	(Ala_{20}→Thr_{778})—(Glu_{1694}→Tyr_{2351})	(Ser_{760}→Thr_{778})—(Glu_{1694}→Arg_{1708})	915

A and B are as defined, supra; "—"represents a peptide bond; "→"indicates a polypeptide sequence inclusive of the specified amino acids; amino acid numbering corresponds to the numbering of the sequence depicted in Table 1; and "deletion"indicates the number of amino acids deleted relative to human factor VII:c.

The procoagulant proteins of the present invention, in addition to lacking a substantial amino acid segment of human factor VIII:C, also have fewer potential N-glycosylation sites than human factor VIII. Preferably, at least one N-glycosylation site has been deleted. More preferably, 18 of the 25 potential N-glycosylation sites are not in the molecule. In still more preferred embodiments, up to 19 of the 25 potential N-glycosylation sites are removed. While not wishing to be bound by theory, it is presently believed that the antibodies to factor VIII:C which are directed to antigenic determinants contained in the protein segment deleted in accordance with this invention, i.e., in the amino acid segement itself or in the carbohydrate portion of the glycosylated protein, will not neutralize the procoagulant proteins of the present invention. Moreover, the fact that the procoagulants of the present invention lack many of the sites for non-human glycosylation by the non-human mammalian or other cells used to produce the proteins is also belived to reduce the antigenicity of that protein, and lessen the likelihood of developing antibodies to the procoagulants. This may enable facilitating the treatment of patients in need of procoagulant therapy.

I contemplate that my compounds can be produced by recombinant DNA techniques at a much lower cost than is possible for production of human factor VIII. The host organisms should more efficiently process and express the substantially simpler molecules of this invention.

The compounds of this invention can be formulated into pharmaceutically acceptable preparations with parenterally acceptable vehicles and excipients in accordance with procedures known in the art.

The pharmaceutical preparations of this invention, suitable for parenteral administration, may conveniently comprise a sterile lyophilized preparation of the protein which may be reconstituted by addition of sterile solution to produce solutions preferably isotonic with the blood of the recipient. The preparation may be presented in unit or multi-dose containers, e.g. in sealed ampoules or vials. Their use would be analogous to that of human factor VIII, appropriately adjusted for potency.

One method by which these proteins can be expressed is by use of DNA which is prepared by cutting a full-length factor VIII:C DNA with the appropriate restriction enzymes to remove a portion of the DNA sequence that codes for amino acids 760 to 1708 of human factor VIII:C. The cut DNA is then ligated with an oligonucleotide that resects the cut DNA and maintains the correct translational reading frame.

Preparation of the cDNA has been set forth in detail in U.S. patent applications Ser. Nos. 546,650 and 644,086, supra. A pSP64 recombinant clone containing the nucleotide sequence depicted in Table 1, designated as pSP64-VIII, is on deposit at the American Type Culture Collection under Accession Number ATCC 39812.

Restriction endonucleases are used to obtain cleavage of the human factor VIII:C cDNA, hereinafter the DNA source sequence, at appropriate sites in the nucleotide sequence. Unless otherwise noted, restriction endonucleases are utilized under the conditions and in the manner recommended by their commercial suppliers. The restriction endonucleases selected herein are those which will enable one to excise with substantial specificity sequences that code for the portion of the factor VIII:C molecule desired to be excised. BamHI and SacI are particularly useful endonucleases. However, the skilled artisan will be able to utilize other restriction

endonucleases chosen by conventional selection methods. The number of nucleotides deleted may vary but care should be taken to insure that the reading frame of the ultimate cDNA sequence will not be affected.

The resulting DNA fragments are then purified using conventional techniques such as those set forth in Maniatis et al., *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory 1982) the disclosure of which is incorporated herein by reference, and *Proc. Natl. Acad. Sci.* 76:615–619 (1979). The purified DNA is then ligated to form the sequence encoding the polypeptide of the preferred invention. When necessary or desirable, the ligation may be within an oligonucleotide that resects the cut DNA and maintains the correct translational reading frame using standard ligation conditions. Ligation reactions are carried on as described by Maniatis et al., supra at 2453–6 using the buffer described at page 246 thereof and using a DNA concentration of 1–100 ug/ml, at a temperature of 23° C. for blunt ended DNA and 16° C. for "sticky ended" DNA. The following double-stranded oligonucleotide is useful when there is BamHI/SacI deletion such as described infra,

5'P-CATGGACCG-3'

3-TCGAGTACCTGGCCTAG 5';

but other oligonucleotides can be selected by the skilled artisan depending upon the deletions made and reaction conditions.

The DNA sequences encoding the novel procoagulant polypeptides can, in addition to other methods, be derived from the sequence of human factor VIII:C DNA by application of oligonucleotide-mediated deletion mutagenesis, often referred to as "loopout" mutagenesis, as described for example in Morinaga, Y. et al. *Biotechnology,* 636–639 (1984).

The new DNA sequences containing the various deletions can then be introduced into appropriate vectors for expression in mammalian cells. The procoagulant activity produced by the transiently transfected or stably transformed host cells may be measured by using standard assays for blood plasma samples.

The eukaryotic cell expression vectors described herein may be synthesized by techniques well known to those skilled in this art. The components of the vectors such as the bacterial replicons, selection genes, enhancers, promoters, and the like may be obtained from natural sources or synthesized by known procedures. See Kaufman et al., *J. Mol. Biol.,* 159: 51–521 (1982); Kaufman, *Proc. Natl. Acad. Sci.* 82: 689–693 (1985).

Established cell lines, including transformed cell lines, are suitable as hosts. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants (including relatively undifferentiated cells such as haematopoeitic stem cells) are also suitable. Candidate cells need not be genotypically deficient in the selection gene so long as the selection gene is dominantly acting.

The host cells preferably will be established mammalian cell lines. For stable integration of the vector DNA into chromosomal DNA, and for subsequent amplification of the integrated vector DNA, CHO (Chinese hamster ovary) cells are presently preferred. See U.S. Pat. No. 4,399,216. Alternatively, the vector DNA could include all or parts of the bovine papilloma virus genome (Lusky et al., *Cell,* 36: 391–401 (1984) and be carried in cell lines such as C127 mouse cells as a stable episomal element. Other usable mammalian cell lines include HeLa, COS-1 monkey cells, melanoma cell lines such as Bowes cells, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cells lines and the like.

Stable transformants then are screened for expression of the procoagulant product by standard immunological or enzymatic assays. The presence of the DNA encoding the procoagulant proteins may be detected by standard procedures such as Southern blotting. Transient expression of the procoagulant genes during the several days after introduction of the expression vector DNA into suitable host cells such as COS-1 monkey cells is measured without selection by enzymatic or immunologic assay of the proteins in the culture medium.

The invention will be further understood with reference to the following illustrative embodiments, which are purely exemplary, and should not be taken as limiting the true scope of the present invention, as described in the claims.

EXAMPLE 1

10 μg. of the plasmid pACE, a pSP64 (Promega Biotec, Madison, Wis.) derivative, containing nucleotides 562-7269 of human factor VIII:C cDNA (nucleotide 1 is the A of the ATG initiator methionine codon) was subjected to partial BamHI digestion in 100 ul containing 50 mM Tris.HCl ph 8.0, 50 mM $MgCl_2$, and 2.4 units BamHI (New England Biolabs) for 30 minutes at 37° C. The reaction was terminated by the addition of EDTA to 20 mM and then extracted once with phenol, once with chloroform , ethanol precipitated and pelleted by centrifugation. DNA was redissolved, cleaved to completion in 50 ul using 40 units SacI for 1.5 hours at 37° C. DNA was then electrophoresed through a buffered 0.6% agarose gel. An 8.1 kb fragment corresponding to the partial BamHI-SacI fragment of pACE lacking only the sequence corresponding to nucleotides 2992-4774 of the factor VIII:C sequence was purified from the gel using the glass powder technique described in *Proc. Nat. Acad. Sci.* 76: 615–619 (1979). Purified DNA was ligated with 100 pmoles of the following double-stranded oligonucleotide

5'P-CATGGACCG-3'

3'-TCGAGTACCTGGCCTAG 5'

using standard ligation conditions. The DNA sequence removed represents the deletion of 584 amino acid sequence beginning with amino acid 998 and continuing through 1581. The oligonucleotide inserted, however, encodes amino acids corresponding to 998–1000. Therefore, the polypeptide encoded contains deletion of 581 amino acids.

DNA was then used to transform competent *E. coli* bacteria, and DNA from several ampicillin resistant transformants was analyzed by restriction mapping to identify a plasmid harboring the desired SacI-BamHI deletion mutant. DNA from this plasmid was digested to completion with KpnI, which cleaves the plasmid uniquely at nucleotide 1816 of the factor VIII:C coding sequence. This DNA was ligated with a KpnI DNA fragment containing nucleotides 1-1815 of factor VIII:C DNA and a synthetic SalI site at nucleotides −11 to −5 and then used to transform competent *E. coli* bacteria.

Plasmid DNA was isolated and oriented by restriction mapping to identify a plasmid, pBSdK, containing the correct 5' to 3' orientation of the KpnI insert. SalI digestion, which excises the entire polypeptide coding region from the plasmid, was performed and the DNA electrophoresed through a buffered 0.6% agarose gel. The 5.3 Kb SalI fragment was purified from the gel as described above. This DNA fragment was ligated with XhoI cut pXMT2 DNA to give rise to plasmid pDGR-2. pXMT2 is a plasmid capable of expressing heterologous genes when introduced into mammalian cells such as the COS-1 African Green Monkey kidney cell line, and is a derivative of the expression vectors described in Kaufman, supra at 689–93. The expression elements are the same as described for plasmid pQ2 except that it contains a deletion of the adenovirus major late promoter extending from −45 to +156 with respect to the transcription start site of the adenovirus major late promoter. mRNA expression in pXMT is driven by the SV40 late promoter. The bacterial replicon, however, has been substituted to render bacteria containing the vector resistant to ampicillin rather than tetracycline. pXMT2 contains a unique Xho I site at a position which allows for expression of inserted cDNA from the SV40 late promoter. This Xho I site is convenient for inserting factor VIII:C cDNA constructs since these are flanked by SalI sites.

Restriction mapping of transformants identified a plasmid, pDGR-2, containing the correct 5' to 3' orientation of the polypeptide coding sequence relative to the direction of transcription from the SV40 late promoter. pDGR-2 is on deposit at the American Type Culture Collection under Accession number 53100.

EXAMPLE 2

Other novel procoagulant proteins may be obtained from constructs produced by oligonucleotide mediated deletion mutagenesis, using for example the "loopout" mutagenesis techniques as described in Morinaga et al., supra. The deletion mutagenesis is performed using expression plasmid pDGR-2 or any other appropriate plasmid or bacteriophage vector. Other methods for oligonucleotide mediated mutagenesis employing single stranded DNA produced with M13 vectors and the like are also suitable. See Zoller et al., *Nucl. Acids Res.* 10: 648–6500 (1982). For example, these deletions can be produced using the oligonucleotides

(A) 5'
AAAAGCAATTTAATGCCACCCCAC-
CAGTCTTGAAACGCCA

(B) 5'
AAAAGCAATTTAATGCCACC-
GAAGATTTTGACATTTATGA

to cause deletions in factor VIII:C cDNA from nucleotides (A) 2334 to 4974 or (B) 2334 to 5079. The proteins encoded by these constructs contain deletions of (A) 880 and (B) 915 amino acids relative to Factor VIII:C.

The deleted constructs are tested directly, or after subcloning into appropriate expression vectors, in order to determine if the novel proteins possess procoagulant activity. Procoagulant activity was assayed as described in Examples 3 and 4.

EXAMPLE 3

Expression of Procoagulant Molecules in COS Monkey Cells The expression plasmids containing the modified cDNA's prepared as in Examples 1 or 2 and the full-length cDNA, pXMT-VIII, were introduced into COS-1 cells via the DEAE-dextran transfection protocol. Sompayrac and Dana 1981, *Proc. Natl. Acad. Sci.* 78: 7575–7578. Conditioned media was harvested 48 hours post-transfection and assayed for factor VIII-type activity as described in Toole et. al., 1984, *Nature* 312:342–347. The results of the experiment are summarized in Table 3. Both plasmids containing the modified cDNAs yielded procoagulant activity and, moreover, the activity was greater than that obtained using wild type cDNA. From these data it was concluded that removal of up to 880 amino acids (95,000 daltons) in a defined domain of human factor VIII does not destroy cofactor activity. Furthermore, these abridged procoagulant proteins retain their ability to be activated by thrombin.

TABLE 3

EXPRESSION OF ABRIDGED FACTOR VIII MOLECULES

plasmid	# amino acids deleted	chromogenic activity ($mUml^{-1}$)	Clotek activity −IIa	Clotek activity +IIa (fold)
No DNA	—	0		
pXMT-VIII	—	15:1	—	450
pDGR-2	581	114	250	5750 (23X)
pLA-2	880	162	330	9240 (28X)

The plasmids indicated were transfected into COS cells and 48 hr. post-transfection the conditioned media taken for assay by the Kabi Coatest factor VIII:C method (chromogenic activity) and by the one-stage activated partial thromboplastin time (APTT) coagulation assay (Clotek activity) using factor VIII:C deficient plasma as described (Toole, *Nature* 1984). For thrombin (IIa) activation, samples were pretreated 1–10 min, with 0.2 units/ml thrombin (IIa) at room temperature. Activation coefficients are provided in parentheses. Activity from media from the wild-type (pXMT-VIII) transfection was too low to directly measure Clotek activity before thrombin activation. From other experiments where the wild type factor VIII activity was concentrated, it was demonstrated to be approximately 30-fold activatable.

EXAMPLE 4

Expression of Procoagulant Molecules in CHO Cells

(A) Expression of pDGR-2

The procoagulant expression vector containing a deletion (relative to the Factor VIII:C cDNA) of 581 amino acids (pDGR-2) was transfected with plasmid pAdD26SV(A)#3 (10 ug pDGR-2:1 ug pAdD26SV-(A)#3) by $CaPO_4$ coprecipitatio CHO DHFR deficient cells (DUKX-B11) and transformants isolated and grown in increasing concentrations of MTX as described by Kaufman et. al., (1985). One transformant designated J1 exhibited the following activities as a function of resistance to increasing concentrations of MTX.

uM MTX	mUnits/ml/day/10^6 cells*
0	1.46
0.02	322
0.1	499

(B) Expression of pLA-2

The procoagulant expression vector containing a deletion of 880 amino acids (pLA-2) was introduced into CHO DHFR deficient cells (DUKX-B11, Chasin and Urlaub, PNAS 77: 4216–4220, 1980 by protoplast fusion as described (Sandri-Goldin et al. Mol. Cell. Biol. 1: 743–752). After fusion, fresh medium containing 100 ug/ml of kanamycin, and 10 ug/ml of each of thymidine, adenosine, deoxyadenosine, penicillin, and streptomycin and 10% dialyzed fetal calf serum was added to each plate. The kanamycin was included to prevent the growth of any bacteria which had escaped conversion to protoplasts. Four days later the cells were subcultured 1:15 into alpha-media with 10% dialyzed fetal calf serum, penicillin, and streptomycin, but lacking the nucleosides. Colonies appeared after 10–12 days after subculturing cells into selective media. A group of 8 transformants were pooled and grown in sequentially increasing concentrations of MTX starting at 0.02 uM with steps to 0.1, 0.2, and 1.0 uM MTX (LA 3–5 cells; ATCC No. CRL 10/01). Results of factor VIII-type activity in cells resistant to increasing concentrations of MTX is shown below.

uM MTX	mUnits/ml/day/10^6 cells*
0	16
0.02	530
0.2	1170
1.0	1890

*Factor VIII activity was determined by the Kabi Coatest factor VIII:C method (chromogenic activity).

What is claimed is:

1. A recombinant DNA which upon expression results in a truncated Factor VIII protein which is an active procoagulant wherein the recombinant DNA encodes for a protein having the amino acid sequence of a human Factor VIII:C except for having a deletion corresponding to at least 581 amino acids within the region between Arg-759 and Ser.-1709, wherein the amino acid numbering is with reference to Met-1 of the human Factor VIII:C leader sequence.

2. The recombinant DNA of claim 1 wherein the deletion corresponds to the region between Pro-1000 and Asp-1582.

3. The recombinant DNA of claim 1 wherein the deletion corresponds to the region between Thr-778 and Pro-1659.

4. The recombinant DNA of claim 1 wherein the deletion corresponds to the region between Thr-778 and Glu-1694.

5. A genetically engineered mammalian host cell containing, and capable of expressing, DNA of claim 1.

6. A genetically engineered mammalian host cell containing, and capable of expressing, DNA of claim 2.

7. A genetically engineered mammalian host cell containing, and capable of expressing, DNA of claim 3.

8. A genetically engineered mammalian host cell containing, and capable of expressing, DNA of claim 4.

9. A method for producing a truncated Factor VIII:C protein which is an active procoagulant having the amino acid sequence of a human Factor VIII:C but lacking at least 581 amino acids of the region between Arg-759 and Ser-1709 which comprises producing a genetically engineered mammalian host cell of claim 5 and culturing said host cell under condition permitting expression of the protein.

10. A truncated human Factor VIII:C protein which is an active procoagulant protein having a peptide sequence of human Factor VIII:C but lacking a peptide region selected from the group consisting of:

(a) the region between Pro-1000 and Asp-1582;

(b) the region between Thr-778 and Pro-1659; and,

(c) the region between Thr-778 and Glu-1694.

11. A pharmaceutical preparation for the treatment of Hemphilia A comprising a sterile preparation containing an effective amount of a protein of claim 9, in admixture with a pharmaceutically accepted carrier.

12. A method for treating Hemophilia A comprising administering to a patient a pharmaceutical preparation of claim 11.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,868,112

DATED : Sep. 19, 1989

INVENTOR(S) : John J. Toole, Jr.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 1, between lines 7 and 8 (before the second paragraph), insert the following:

-- This invention was made with Government support under DHHS grant S R44 HL35946-03 awarded by the NIH. The Government has certain rights under the invention. --.

Signed and Sealed this

Third Day of November, 1992

Attest:

DOUGLAS B. COMER

Attesting Officer

Acting Commissioner of Patents and Trademarks

*** APPLICATION INFORMATION DISPLAY ***

04/27/00 15:31 DETAIL INFORMATION:

SC/SN: 07/010085
FILDT: 04/11/86
PATNO: 4868112 PUBNO: I103215
ISSDT: 09/19/89 PUBDT: 00/00/00
ABNDT: 00/00/00 PGPUB CL/SC: / .
APPL: TOOLE
LOC: 4300 LOCDT: 09/09/99 BATNO: 000
CHG-LOC: IE TEAM: 00 ISSNO: 38
CHGTO-NAME: NO NAME FOUND
TOT ACT: 05 STATUS: 174 STADT: 01/05/94
RESP CD: MISC START DT: 07/23/93 DUE DT: 10/25/93
EXMR NO/NAME: 68940/SISSON, BRADLEY L
DOCKET DATE: 07/23/93 GAU: 1655 L R CD: 01
ATTY DOCK #: 5031-A-PCT LOST N LOST DT 00/00/00
APPLN TYPE: 1 TYPE SM ENT: 0 UNMAT PET: N
CURR CL/SC: 435/069.600 FOR PRIOR CL: N PET FAOM:
TITLE OF INVENTION: UNAVAIL FOR ACTION: N PP UNAVAIL:
NOVEL PROCOAGULANT PROTEINS

CONTENTS:

27	DOCK	D	02/17/00
26	DOCK	D	09/18/99
25	DOCK	D	04/16/99
24	DOCK	D	11/15/97
23	DOCK	D	10/05/96
22	I.D.	O	01/05/94
21	CTIN	E	07/29/93
20	MAIL	O	07/23/93
19	CTMS	O	07/23/93
18	DOCK	D	07/23/93
17	N423	C	09/29/92
16	PGM/	O	12/13/89
15	N084	B	07/20/89
14	N/=.	N	04/18/89
13	CNTA	A	04/14/89
12	EXIN	O	03/28/89

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END OF DISPLAY TO DISPLAY CONTENTS: PUSH SEND

Patent Maintenance Fees - Public Inquiry

Patent#: 4868112 Filed: 04/11/86 Issued: 09/19/89 Serial#: 07010085
Status: 12th Year Fee Window Opens: 09/19/00 Sml Entity: NO
Window Opens: 09/19/00 Surchg Due: 03/19/01 Expiration: 09/19/01
Fee Amt Due:$ 2910 Surchg Amt Due:$ Total Amt Due:$ 2910
Fee Code: 185 Surchg Code:
Title: NOVEL PROCOAGULANT PROTEINS

Address For Fee Purposes:
BRUCE M. EISEN
GENETICS INSTITUTE, INC.
87 CAMBRIDGEPARK DRIVE
CAMBRIDGE MA 02140

Most Recent Significant Events:

03/19/97	Payment of Maintenance Fee, 8th Year, Large Entity
03/25/93	Payor Number Assigned
03/15/93	Payment of Maintenance Fee, 4th Year, Large Entity
03/15/93	Last Event On Maintenance History

Chronological Record of Genetics Institute's and Pharmacia & Upjohn's ReFacto® Antihemophilic Factor (Recombinant) [r-VIII SQ] BB-IND 5348 Submissions

Date of Submission (mm-dd-yy)	IND Serial No.	Summary of Contents
11-30-93	000	ReFacto Hemophilia A IND Original Submission
03-23-94	001	Relocation Of Kabi Pharmacia
04-08-94	002	Clarification Requested Of 3-11-94 Clinical Hold Letter
05-03-94	003	Response To First 4 Comments Of Clinical Hold Letter
05-10-94	004	Mink Sl Focus Induction Assay
06-22-94	005	Mouse Antibody Production Assay
07-06-94	006	Separation/Inactivation Of Murine Xenotropic Retrovirus
07-21-94	007	Final Report: Process Validation Of Removal Or Inactivation Of Murine Xenotropic Retrovirus From Spiked Material
09-06-94	008	Request For FDA Meeting And Proposed Agenda
10-19-94	009	Minutes From 9-23-94 Meeting With FDA
10-31-94	010	Response To 3-14-94 FDA Questions-Requests For Information
11-01-94	011	Response To 3-14-94 FDA Questions/Request For Information
11-09-94	012	Response To FDA Questions
11-17-94	013	Notification Of Update Of Master File For Production Of Bulk Purified 8a4 MAB
12-02-94	014	Response To FDA Request For Information: PTP Surgery And PUP Protocols
12-07-94	015	Clinical Development Plan
12-14-94	016	Proposed Agenda For 12-21-94 Meeting

Date of Submission (mm-dd-yy)	IND Serial No.	Summary of Contents
12-14-94	017	Differences In European And U.S. Phase III Study Protocols For PTP, PUP, Surgery Studies
12-19-94	018	Master Schedule For Viral Testing Used During Production Of R-VIII SQ
02-01-95	019	Minutes Of 12-21-94 Meeting With FDA
02-15-95	020	Lab Tests For Safety Assessments In PUPs
02-16-95	021	Response To CMC Questions
02-20-95	022	CMC Data For Method D Product
03-02-95	023	Update Of Equipment And Facilities Portions Of The Cell Culture And Purification Process Sections Of The IND
03-09-95	024	Revised PTP Protocol 93-R831-013
03-30-95	025	Revised PUP Protocol 93-R833-019
04-11-95	026	Discussion Of Significance Of Production Doubling Number
05-18-95	027	Revised PTP, PUP and Surgery Protocols 93-R831-013, 93-R833-019 and 93-R832-020
07-31-95	028	New Investigators Revised CIB
08-01-95	029	Correction Of Misidentification Of Formulations Used In Various Protocols
09-05-95	030	Changes In PTP Surgery And PUP Protocols 93-R831-013 93-R833-019 and 93-R832-020
09-12-95	031	New Investigators
09-19-95	032	Final Draft Of Protocol For 3-Way Crossover PK Study Ctn 95-R811-057
11-01-95	033	New Investigators
12-05-95	034	Clinical Bibliography Map Assay, PK Reports 9496117 and 9496118

Date of Submission (mm-dd-yy)	IND Serial No.	Summary of Contents
01-05-96	035	New Investigators
02-07-96	036	Protocol For 3-Way Crossover PK Study 95-R811-057
02-29-96	037	New Investigators
04-01-96	038	New Investigators
04-25-96	039	New Investigators
06-11-96	040	Revision In 3-Way PK Protocol CTN 95-R811-057
06-26-96	041	Transfer Of Ownership From Pharmacia To Pharmacia And Upjohn Company
07-08-96	042	Annual Report
07-01-96	043	New Investigators
07-17-96	044	Revision In Surgery Protocol 93-R831-013, New Investigators
08-16-96	045	Revision In PUP Protocol 93-R833-019, New Investigators
09-03-96	046	Safety Report: Hematoma
09-05-96	047	Safety Report: Inhibitor Development
09-26-96	048	Proposal For BLA Containing Modified Patient Numbers And Demographics
11-19-96	049	New Investigators, 36-Mo Stability Info, Method C
11-25-96	050	Safety Report: Inhibitor Development
12-31-96	051	New Investigators
01-31-97	052	Safety Report: Anaphylaxis
01-31-97	053	Fax Re: 10-Day Safety Report
03-05-97	054	Non-Evaluability Of PK Data From Protocols 93-R831-013 And 93-R833-019

Date of Submission (mm-dd-yy)	IND Serial No.	Summary of Contents
03-12-97	055	Safety Report: Acute Renal Failure
04-07-97	056	Tox Data (Paravenous And Intra-arterial Tolerance Study In The Beagle Dog)
05-20-97	057	Annual Report
05-30-97	058	Investigator Information And Updated Stability
06-06-97	059	Response To Questions
07-01-97	060	Change In Investigator Site
09-03-97	061	10-Day Safety Report: Inhibitor
09-15-97	062	10-Day Safety Report: Inhibitor
10-21-97	063	Follow-Up To Amendments 061 And 062
11-06-97	064	Transfer of IND Ownership (P&U Letter)
11-06-97	065	Transfer of IND Ownership (GI Letter)
11-07-97	066	Request For Meeting
12-03-97	067	Pre-Meeting Materials
01-15-98	068	Raw Material Sourcing Information requested by the FDA
03-25-98	069	Clinical Labeling Revision
05-08-98	070	Clinical Labeling Revision
05-27-98	071	IND Safety Report (15-Day)
08-27-98	072	IND Safety Report (15-Day)
09-11-98	073	IND Safety Report (15-Day) follow-up
10-01-98	074	1997 IND Annual Report
10-05-98	075	IND Safety Report (15-Day) follow-up

Date of Submission (mm-dd-yy)	IND Serial No.	Summary of Contents
10-20-98	076	IND Safety Report (15-Day)
10-22-98	077	Clinical Labeling Revision
11-05-98	078	Information Amendment: Chemistry, Manufacturing and Controls
11-20-98	079	Information Amendment: Chemistry, Manufacturing and Controls
12-04-98	080	IND Safety Report (15-Day)
02-26-99	081	IND Safety Report (15-Day)
03-12-99	082	IND Safety Report (15-Day)
03-25-99	083	IND Safety Report (15-Day)
03-26-99	084	IND Safety Report (15-Day)
04-20-99	085	IND Safety Report (15-Day)
05-06-99	086	IND Safety Report (15-Day)
11-24-99	087	IND Safety Report
11-29-99	088	IND Safety Report
01-27-00	089	IND Safety Report (15-Day)
01-31-00	090	1998 IND Annual Report

Chronological Record of Genetics Institute's , ReFacto® Antihemophilic Factor (Recombinant) [r-VIII SQ] Biologics License Application Submissions (Ref. No. 98-0137)

Date of Submission (mm-dd-yy)	BLA Serial No.	Summary of Contents
02-02-98	000	Biologics License Application, ReFacto® Antihemophilic Factor (Recombinant) [r-VIII SQ]
07-10-98	001	Response to FDA Questions Dated May 15, 1998
09-18-98	002	CMC Information
10-26-98	003	Response to Request for Draft Labeling
11-10-98	004	Response to Requests for Additional Information
12-08-98	005	Revised Draft Package Insert
12-08-98	006	Response to FDA Request for Data
12-15-98	007	Response to Request for Revisions to Draft Labeling
12-24-98	008	CMC Information
12-24-98	009	CMC Information
01-05-99	010	CMC Information
01-14-99	011	Revision to Draft Vial Label
02-08-99	012	Notice of Intent to File Amendment
04-02-99	013	Teleconference Record
04-06-99	014	Company Responses to FDA Complete Response Letter
05-13-99	015	Company Responses to FDA Complete Response Letter
06-04-99	016	Company Responses to FDA Complete Response Letter
06-16-99	017	CMC Information
06-21-99	018	Request for Meeting
06-24-99	019	Draft Vial and Carton Labels

Date of Submission (mm-dd-yy)	BLA Serial No.	Summary of Contents
07-08-99	020	CMC Information
07-13-99	021	Pre Meeting Package
08-04-99	022	Communication Authorization HPB-FDA
09-24-99	023	Request for meeting
10-14-99	024	Pre-meeting Materials
10-28-99	025	Meeting Agenda and Participants
11-09-99	026	Draft Vial and Carton Labels
11-10-99	027	Ethylene Glycol Specification Request and Updated Active Substance Specific Activity Specification
11-16-99	028	Follow-up Data Requested By CBER During Prophylaxis Indication Meeting
11-17-99	029	Responses to Questions Received from CBER on October 12, 1999
11-22-99	030	Meeting Package
11-24-99	031	Virus Removal Validation Data for HSA
12-20-99	032	Minor updates to CMC information
12-21-99	033	CMC Information
01-27-00	034	Draft Package Insert
01-28-00	035	Draft Summary Basis for Approval
02-16-00	036	Revised Labeling: Draft Package Insert
02-29-00	037	Final Surgery Report
02-29-00	038	Post-licensure commitments
03-02-00	039	Post-licensure commitments

Date of Submission (mm-dd-yy)	BLA Serial No.	Summary of Contents
03-02-00	040	Revised Labeling: Draft Package Insert
03-03-00	041	Prophylaxis Surgery Study Commitment
03-03-00	042	Revised Labeling: Draft Package Insert
03-06-00	043	Revised Labeling: Draft Package Insert
03-06-00	044	Prophylaxis Study Commitment

Chronological Record of Genetics Institute's Significant FDA Meetings concerning ReFacto® Antihemophilic Factor (Recombinant) [r-VIII SQ]

Date of Meeting (mm-dd-yy)	FDA Contact	Summary
12-10-97	CBER	Pre-BLA Meeting
11-23-98	CBER	Discussion of the ReFacto Assay
12-11-98	Blood Products Advisory Committee	ReFacto Overview
07-22-99	CBER	Prophylaxis Indication
11-04-99	CBER	ReFacto and US Sourced HSA/ReFacto from the Modified Process

CBER = Center for Biologics Evaluation and Research

Chronological Record of Genetics Institute's and Pharmacia & Upjohn's Submissions to Office of Orphan Products Development, FDA, concerning Orphan Drug Status for ReFacto® Antihemophilic Factor (Recombinant) [r-VIII SQ]

Date of Submission (mm-dd-yy)	Summary of Contents
12-14-94	Minutes of 11-21-94 Orphan Drug meeting
11-20-95	Request for Orphan Drug Designation [granted 02-08-96]
02-15-96	Response to FDA request for information
10-22-96	Request for a meeting
04-25-97	Annual Report
11-12-97	Transfer of Ownership of Orphan Drug Designation
04-23-98	1997 Annual Report
08-20-98	Orphan Drug considerations relating to ReFacto®
09-18-98	Fax containing documentation of shortages of FVIII
11-24-98	Fax of 12-10-98 Blood Products Advisory Committee Meeting to discuss ReFacto and recommendations #83 and #89 of the Medical and Scientific Advisory council of the National Hemophilia Foundation
11-25-98	Recall of Alphanate
11-30-98	Fax of Draft Orphan Drug Section in BPAC Pre-meeting package
12-17-98	Recall of Koate
01-06-99	Hold of Kogenate in Canada
01-22-99	Log of GI-Bayer contacts concerning cross licensing of hemophilia A products
02-24-00	1998-99 Annual Report
02-29-00	Letter requesting marketing exclusivity
02-28-00	Amendment to Orphan Drug Designation

Chronological Record of Genetics Institute's Significant FDA Telephone Contacts Concerning ReFacto® Antihemophilic Factor (Recombinant) [r-VIII SQ]

Date (mm-dd-yy)	FDA Contact	Purpose
12-16-97	T. Lachenbruch	Pre-BLA Meeting Follow-up re Electronic data
02-06-98	M. Padgett	Additional Copies of SAS Data Sets
03-24-98	M. Serabian	Extent of Preclinical data on file
05-15-98	A. Chang	CMC Questions
07-01-98	C. Cary	Manufacturing Schedules for P&U
07-01-98	A. Chang	Nature of FDA Concern re: HSA Sourced From European Donors
07-17-98	R. Darius	Clarification of BLA Number
07-21-98	A. Chang	Refacto IND Annual Report Extension
07-23-98	A. Chang	Response to 7/21 Questions
08-04-98	A. Chang	Set up the appropriate assays for ReFacto
08-10-98	B. Darius	Information promised on 7/17/98
08-31-98	A. Chang	Ground transportation
09-04-98	S. Donahoe	Inquiry into status of petition of August 20 to OPD regarding orphan drug considerations affecting Refacto
09-10-98	A. Chang	Fax from P&U regarding arrangements
09-17-98	A. Chang	Inspection Issues
09-18-98	A. Chang	Expect Clinical and additional CMC BLA Review Questions
09-22-98	L. Wood	Chromogenic Assay
09-24-98	M. Padgett	Follow up requests from Mary Padgett
09-25-98	R. Darius	Arrangements for P & U Facility Inspection
10-01-98	R. Darius	Production Schedule for P&U Inspection

Date (mm-dd-yy)	FDA Contact	Purpose
11-03-98	S .Donahoe	Inquiry into status of petition of August 20 to OPD regarding orphan drug considerations affecting Refacto
11-13-98	A. Chang	90:1 to 90:2 Ratios
11-17-98	M. Padgett	Request for additional clinical volume 1 copies, BPAC details, date to discuss vial/carton labels
11-24-98	A. Chang	BPAC, Orphan Drug, HSA Sourcing issues
11-30-98	J. McCormick	Inquiry concerning Mccormick's presentation at upcoming BPAC re ReFacto and Kogenate exclusivity
12-01-98	M. Padgett	ReFacto vial and carton labels
12-03-98	R. Pierce	Matched Pair Analyses for BPAC
12-04-98	A. Chang	Data Comparing Potency of 37 batches
12-07-98	Dr. Green	Summary of 12-04-98 conversation
12-07-98	J. McCormick	Second Inquiry concerning McCormick's presentation at upcoming BPAC re ReFacto and Kogenate exclusivity
12-08-98	R. Pierce	SAS data listings
12-17-98	J. McCormick	Inquiry concerning date certain for Bayer's response to OPD concerning shortages
12-23-98	A. Chang	Amendments to BLA
01-01-99	A. Chang	ReFacto licensing items
01-11-99	M. Padgett	ReFacto Labeling
01-19-99	M. Padgett	ReFacto Labeling- Vial Peel Off
01-20-99	M. Padgett	ReFacto Labeling- Vial Peel Off
01-21-99	S. Risso	Comparability Protocol for New Facility
01-25-99	J. Eltermann	Suite A E.coli to CHO and ReFacto St. Louis

Date (mm-dd-yy)	FDA Contact	Purpose
01-25-99	J. Eltermann	Suite A E.coli to CHO and ReFacto St. Louis
01-26-99	A. Chang	ReFacto Licensing Issues
01-27-99	J. McCormick	Request for teleconference call on Friday Jan 29
03-26-99	P. Aebersold	Clarification of Question 7, pt. number 45-132 of the Complete Response Letter
04-06-99	M. Padgett	Prophylaxis questions for Complete Response Letter
04-12-99	A. Chang	Feedback on Plasma Sourcing Submission
05-18-98	A. Chang	Fax re: HSA sourcing issues
05-28-99	M. Padgett	Feedback on 5/18/99 fax to Andrew Change re: HSA sourcing
06-03-99	M. Weinstein	Follow up clarification re: HSA
06-08-99	M. Padgett	Restart of review clock, prophylaxis meeting with FDA
06-14-99	D. Parshall	Conformance Lots
06-14-99	M. Padgett	Fax: HSA Teleconference materials
06-15-99	T. Lynch	Teleconference: Review of GI's fax (6/14/99) on HSA Options
06-18-99	A. Chang	ReFacto HSA Sourcing
06-22-99	M. Padgett	Shipment of Vials for Ethylene Glycol Testing
07-15-99	M. Padgett	ReFacto prophylaxis indication meeting, attendees, pre-meeting package
07-19-99	A. Chang	Double Pasteurized Albumin & ReFacto Diluent
09-20-99	M. Padgett	Extension of IND annual report until end of December 1999
10-07-99	M. Padgett	BLA Review Comments
10-12-99	M. Padgett et al	FDA clinical questions from 10-12-99 teleconference
10-15-99	K. Towns	Request for additional vials for conformance lot testing

Date (mm-dd-yy)	FDA Contact	Purpose
10-20-99	A. Chang	Fax: Fill areas shared with albumin
10-27-99	A. Chang	Conformance Lot Information
10-28-99	M. Padgett	CBER Attendees for ReFacto US sourced HSA, ReFacto Plus meeting
11-05-99	A. Chang	HSA/ReFacto+ Meeting Summary
11-10-99	M. Padgett	Request for Meeting, Medical Policy Coordination Committee
11-10-99	M. Padgett	Second set of Conformance lots
11-16-99	M. Padgett	Follow-up on Meeting Request
11-23-99	A. Chang	Confirming HSA Submission
11-23-99	A. Chang	Follow-up HSA
11-24-99	A. Chang	Fax: ICH Residual Solvent Guideline
11-24-99	A. Chang	Ethylene glycol testing
11-30-99	M. Padgett	Major Amendment Letter
12-15-99	M. Padgett	Resolution of HSA Issue / Conformance Lots
12-23-99	J. Capen	Extension of IND annual report until end of January 2000
01-11-00	A. Chang	Specifications and other CMC information
01-27-00	M. Padgett	ReFacto PI draft proposed revision for negotiation with FDA
01-28-00	M. Padgett	ReFacto Summary of Basis for Approval draft proposed revision for negotiation with FDA
02-09-00	M. Padgett	ReFacto PI negotiation licensing issues
02-11-00	M. Padgett et al	ReFacto PI negotiation
02-16-00	M. Padgett	ReFacto PI submission date, post licensing commitments, exemption from lot release
02-17-00	M. Padgett	Additional color copies of ReFacto PI

Date (mm-dd-yy)	FDA Contact	Purpose
02-22-00	M. Padgett	Schedule teleconference for PI negotiation
02-25-00	M. Padgett et al	ReFacto PI negotiation
02-28-00	M. Padgett et al	ReFacto PI negotiation
03-02-00	M. Padgett	Revised ReFacto package insert
03-03-00a	M. Padgett	Revised commitment letter for ReFacto
03-03-00b	M. Padgett	Revised package insert for ReFacto BLA 98-0137.042
03-06-00	M. Padgett et al	ReFacto PI negotiation

PATENT
Atty. Docket No.: 01142.0130

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 4,868,112)
)
Issued: September 19, 1989)
)
To: John J. Toole, Jr.)
)
Assignee: Genetics Institute, Inc.)
)
For: NOVEL PROCOAGULANT PROTEINS)

BOX PATENT EXT.
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

CERTIFICATION

I, STEVEN P. O'CONNOR, do hereby certify that this accompanying application for extension of the term of U.S. Patent 4,868,112 under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and four (4) copies thereof.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

By: Steven P. O'Connor
Reg. No. 41,225

Date: May 4, 2000

LAW OFFICES
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202-408-4000

PATENT
Atty. Docket No.: 01142.0130

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 4,868,112	)
	)
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Assignee: Genetics Institute, Inc.	)
	)
For: NOVEL PROCOAGULANT PROTEINS	)

BOX PATENT EXT.
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION ACCOMPANYING APPLICATION UNDER
35 U.S.C. § 156 FOR EXTENSION OF PATENT TERM

I, STEVEN P. O'CONNOR, do hereby declare:

I am a patent attorney authorized to practice before the United States Patent and Trademark Office and I have been appointed as an attorney by the patent Assignee, Genetics Institute, Inc., with regard to this application for extension of the term of U.S. Patent No. 4,868,112 and to transact all business in the U.S. Patent and Trademark Office in connection therewith.

I have reviewed and understand the contents of the accompanying application being submitted pursuant to 37 C.F.R. § 1.740.

I believe that the patent is subject to extension pursuant to 37 C.F.R. § 1.710.

LAW OFFICES
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I believe an extension of the length claimed is justified under 35 U.S.C. § 156 and applicable regulations.

I believe the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 37 C.F.R. § 1.720.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

By: Steven P. O'Connor

Steven P. O'Connor
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Date: May 4, 2000

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